The Influence of Pollinator Diversity and Behaviour on Pollen Movement in *Brassica rapa chinensis* (Pak-Choi) Crops, and its Significance for Gene Escape

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ABSTRACT

The overall aim of the study was to assess the risk of gene flow from *Brassica* crops by insect-mediated pollen transport. I measured the viability of pollen in *Brassica* flowers throughout crop development and compared this with the viability of pollen transported by insects inside and outside one early- and one late-season crop. In order to evaluate the relative importance of different species in pollen transport, I measured abundance of flower visitors during crop development, and measured the foraging behaviour of five key pollinator species throughout the growing season, in relation to variation in microclimate, crop phenology and the relative abundance of other pollinator species competing for flower resources.

Flower visiting insects of *Brassica rapa* crops were highly diverse, and their abundance and diversity changed with crop phenology. I found similar abundances at the family level for both crops studied, although capture rates were greater in the early- than in the late-season crop. Across flowering development, the greatest numbers of insects were captured at the peak of flowering for both crops. During the flowering period, Diptera was the most abundant order collected, followed by Hymenoptera. The most abundant family in Hymenoptera was Apidae which tracked crop development in both fields, with greater numbers of insects captured inside than outside the field. Standardized-count pollen loads were smaller in Diptera than in Hymenoptera. Of the five key pollinator species sampled, *Lasioglossum sordidum* (Hymenoptera: Halictidae), *Apis mellifera* (Hymenoptera: Apidae) and *Bombus terrestris* (Hymenoptera: Apidae) transported similar pollen loads, which were much greater than those carried by *Eristalis tenax* (Diptera: Syrphidae) and *Melangyna novae-zealandiae* (Diptera: Syrphidae).

The numbers of insects captured outside of the crop were 10% and 33% of the totals captured inside for the early- and the late-season crop, respectively. The proportion of insects entering versus leaving the crop varied considerably across species, crops and trap location (i.e., whether traps were inside or 50 m outside the border of the crop). However, it is worth noting that not uncommonly more insects were attracted into the crop early in the season, staying there rather than leaving, and then when flowers started to disappear there was a massive escape of insects leaving.

This research provides evidence for the influence of crop age on the foraging behaviour of key pollinators and for species-specific variation in the foraging behaviour of *Brassica* visitors with crop development. Temporal variation in the rate and variability of movement between

flowers, and the duration and variability in time spent on each flower, throughout the growing season differed markedly between pollinator species. Flower density, plant density, and the abundance of other insects contributed to the observed variation in pollinator behavioural activity for *A. mellifera*, *E. tenax*, *M. novae-zelandiae* and *L. sordidum*.

Bombus terrestris had the greatest rates and variability of movement, and the greatest rates of flower visitation among all key pollinators studied. Therefore *B. terrestris* might contribute to gene flow to a greater extent than other key pollinators. Additionally *B. terrestris* had the greatest variability in the rate of movement, increasing the risk of pollen movement over long distances.

In summary, I found that (i) insect abundance and diversity changed with crop phenology and Diptera was the most abundant order collected, (ii) flower density, plant density, and the abundance of other insect pollinators were important factors explaining pollinator behaviour for all key pollinators, except *B. terrestris*, (iii) *B. terrestris* might contribute to gene flow to a greater extent than other key pollinators, because it has a greater rate of flower visitation and a greater flight distance between flowers than other pollinators, and (iv) pollen viability tended to decrease with crop development and declined sharply even just 50 m outside the edge of the crop.

Key words: behaviour, *Brassica rapa*, gene flow, pollen viability, pollinator diversity.

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Chapter One
Introduction

The great 'GM' debate in modern agriculture

The techniques for developing new and improved crop cultivars in agriculture have changed dramatically in the last 20 years. Traditional plant breeding approaches rely on cross pollination to create new varieties (Ellstrand & Hoffman 1990). In contrast, modern plant biotechnology identifies and isolates the functional actions of genes in one organism, and then inserts these genes into another organism, with the goal of expressing novel, desirable traits in a target crop (Messeguer 2003). These cultivars have become known as genetically modified (GM), or genetically engineered (GE), crops. The first experimental planting of GM crops dates back to 1986, and increased progressively to 690 million hectares worldwide in 2003 (James 2007). By 2005, field trials had been conducted for more than 4000 GM cultivars worldwide, from which only 40 transgenic crops have been released for commercial purposes (Daniell 1999, Brookes & Barfoot 2005). China, Canada, Argentina and the US are the main countries that have developed GM crops (James 2003). The typical argument for promoting GM crops is the necessity to improve the efficiency of crop production, because of continued increases in human population and reduction in available land suitable for growing crops (FAO 1996). Other benefits are derived from the reduced amounts of herbicides and insecticides used in GM crops compared to unimproved crops (Bennett *et al*. 2004), increased salt tolerance, improved resistance to disease, and better prevention of soil degradation, leading to higher crop productivity and associated increases in human health due to nutritionally-improved crops (Culpepper & York 1998, Schnepf *et al*. 1998, Silvers *et al*. 2003, Thies & Devare 2007). Finally several authors argue that GM crops will enhance the welfare of future generations without significant adverse impacts (James 2003).

However, the development of GM crops has been highly controversial because of concerns about potential risks to the environment and human health (Trewavas 1999, Dale *et al*. 2002, Bodulovic 2005). These concerns have greatly limited public acceptance of GM crops in many countries, such that stringent evaluation of the possible impact of a new GM cultivar on food, human or animal health, or on the environment is required prior to commercialisation and release (Dale 1999, 2002). Some authors consider that GM plant technology has received remarkably rigid regulation compared to genetic 'improvement' of crops using traditional methods (Poppy 2000), to the point that the cost of regulatory requirements and commercial restriction has become an obstacle to development of transgenic crops (Singh 2006). For example, the European Union holds the position that the consumer

should have the choice whether or not to consume a GM plant, or a derivative of a GM product, requiring strict labelling that has increased the cost of commercialisation (Caswell 2000).

Public concern stems from a range of issues, including the potential for antibiotic selection markers in transgenic food to inactive oral doses of antibiotics, or the potential to promote the spread of pathogens that are resistant to antibiotic treatment (Daniell 1999, Daniell *et al*. 2001). Environmental concerns include the persistence of crop residues in the environment, the development of insecticide or herbicide resistance in pest populations, and unfavourable effects on non-target organisms through processes such as transgene movement into wild plants (Firbank *et al.* 2005, Thies & Devare 2007). In insect-pollinated crop plants, in particular, transgenes can be transported long distances from crops to wild plants or related weed species (Pimentel *et al.* 1989, Hoffman 1990, Chevre *et al*. 2003, Firbank *et al*. 2005). The potential movement of transgenes from GM crops to non-GM crops via insect-mediated pollen dispersal has been highlighted as one of the areas of greatest concern with GM crops (Hüsken & Dietz-Pfeilstetter 2007), and is the motivation behind testing the relative importance of different insect species in dispersing viable pollen into and out of crops in this thesis.

Gene flow and the importance of pollen movement by insects

The term gene flow has been defined as a composite term encompassing all mechanisms resulting in the movement of genes from one population to another (Slatkink 1985, Légère 2005). There are two broad pathways for genes transfer between individuals or populations: “horizontal transfers”, in which the movement of genetic material (by humans) is independent of regular reproductive mechanisms (Berllota & Simonet 1999, Ochman *et al*. 2000) and “vertical transfers” in which gene flow occurs from parent to offspring through cross-pollination within or between crops, or with related weed or native plants (Pylatuik *et al*. 1988, Kidwell 2002).

In flowering plants, vertical gene flow can redistribute genes in the landscape through pollen dispersal prior to fertilization (Ellstrand 1989, Brown & Brown 1996), or through seed dispersal by natural vectors, such as wind, water or animals (Price *et al.* 1996). Pollen grains can be dispersed by natural vectors such as wind or animals (e.g., birds, insects or bats) (Free 1970), and it has been reported that animals (particularly insects) are more efficient pollinators than wind pollination, because the pollen arrives specifically at the stigma, rather than being dispersed into the environment at random (Hayter & Cresswell 2006). A wide range of insects, including honey bees, flies, butterflies and many other insect species are

important in the pollination process (Eastham & Sweet 2002). The overwhelming importance of insects in crop pollination is exemplified by the degree to which humans have bred and manipulated colonies of honey bees to provide crop pollination services 'on demand' (Williams *et al*. 1993). For example, Mesquida *et al*. (1988) showed that the presence of bees results in increased crop yield, because of an increased frequency of successful fertilisation taking place. The success of insect pollination is determined by the structure of the flower, the ability of flower-visiting insects to transfer pollen, and also by the foraging strategy of the pollinator species, which can be dependent on the age, density and structural attributes of the crop (Levin & Kerster 1969). Consequently, the risk of transgene movement out of a crop will also be strongly influenced by pollinator behavioural activity patterns, and pollen 'carry over' from flower to flower by insects, as well as on the abundance of closely-related weed species in the surrounding landscape, the overlap in the flowering periods between GM and non-GM crops, and a range of abiotic variables (Arias & Rieseberg 1994). Perhaps the least understood of these processes is the role of species-specific differences in the behavioural activities of pollinators in determining patterns of pollen movement throughout the phenological cycle of the crop.

Pollinators are principally attracted to flowers as a food source, and use colour, shape and odour cues to recognize flowers (Free 1970). Searching for nectar and pollen resources entails costs associated with the energy expended on movement and increased risk of predation (Harder *et al*. 2001). The relative costs and benefits of obtaining food resources influence the number of flowers visited and the rate movement from plant to plant (Heinrich 1975). Pollinators in their search to be efficient have to choose not only between individual plants, but also between different patches of plants of differing abundance and quality in the landscape (Goulson 1999), maximizing the rate of energy harvesting while minimizing inter-floral flight distance (Pyke 1978, Waser 1982). The flight distance by a pollinator is dependant on crop density and increases as plant density decrease (Levin & Kerster1969)

Brassica as a 'model' system to study variation in pollinator activity with changing crop phenology

The genus *Brassica* contains a diverse group of species of great economic value, providing edible raw vegetables, sources of condiment mustard, edible and industrial oil, animal fodder, and green manure (Williams & Hill 1986). Commercial fields of *Brassica* are mainly sown in autumn flowering in spring, or sown in spring flowering in summer (Williams 1985). *Brassica* flowers have colourful petals, large amounts of pollen, significant scent production and they maintain nectar production during the whole flowering period, which attracts a wide

range of insect pollinators (Free 1970). The Canterbury region in New Zealand produces many seed crops of Brassicacea, including cultivars such as *B. napus oleifera* (Canola, Oilseed rape), *B. rapa chinensis* (Pak choi, chinese mustard), *B. oleracea* (cabbage), *Raphanus sativus* (radish) and *Sinapsis alba* (White Mustard) (Stewart 2002). *Brassica rapa*, in particular, is a crop well suited for research, because it has a fast life cycle, it does not self pollinate, and it requires insects or wind for cross pollination (Stewart 2002). *Brassica rapa* crops also produce large amounts of pollen (9.3 ± 0.5 kg pollen ha^{-1}) (Westcott & Nelson 2001) which makes the investigation of gene flow by pollen movement somewhat easier (Légère 2005, Ceddia *et al*. 2007).

Brassica rapa has bright green foliage and branches that originate in the axis of the highest leaves on each stem and terminate in an inflorescence. The inflorescence is an elongated raceme with shiny yellow flowers, with flowers that stand well above the unopened younger buds. *Brassica rapa* is an obligate outcrosser, due to the presence of self-incompatible genes (Downey *et al*. 1980). Cross pollination in flowering plants depends on the distance between conspecific plants, the number of flowers on the plant, the amount of food in each flower, the distance the insect can travel, and the amount of food the insect can collect (Raw 2000).

Williams (1985) reported that there are differences in the abundance of pollinator species as a function of the timing of flowering in *Brassica* crops, which can be in either spring or summer. Studies have reported that *Brassica* crops are extremely attractive to many flower visitors in different taxa, including Diptera, Lepidoptera, Coleoptera, solitary bees such as *Leioproctus* and *Lasioglossum* and social bees from the genera *Apis* and *Bombus* (Brunel *et al.* 1992, Donovan 1980, Chaudary 2001, Goodell & Thomson 2006). However, the different pollinator species vary greatly in their size and flight capabilities, such that the relative activity rates of insect species can vary markedly across different spatial scales and throughout the growing season (Firbank *et al.* 2003, Hayter & Cresswell 2006, Shali & Conner 2007), which may have important implications for the risk of gene flow. Some studies have detected insect movement of pollen 2.5 km from the source, and the pollen was still viable and able to produce seed (Timmons *et al*. 1995). Rieger *et al*. (2002) reported pollen flow up to 3 km from the source and others authors have reported a wide range of different distances of gene flow for different species (Beckie *et al.* 2003). Average flight distances of different species also appear to change during the season, as crop flowering phenology changes. For example, Kwak (1997) reported that in the case of *Bombus terrestris* the longest distance flown was observed during early flowering and therefore most pollen flow should be expected at this stage. However different results have been found by other authors.

Stephenson (1982) found that *Catalpa speciosa* (Bignoniaceae) flowers that opened late in the season had the greatest chance of being cross pollinated, while Carpenter (1976) reported that out-crossing is more likely to occur in very early and very late flowering, while Heinrich (1975) found that out-crossing was greatest at the peak of the flowering process.

The relative importance of pollinator species in transporting viable pollen

The total pollen load carried by pollinating insects varies greatly between species (Conner *et al*. 1995), although it is closely correlated with body size (Adler & Irwin 2005). Free & Williams (1972) pointed out that the ability to carry pollen is also related to species-specific qualitative traits, such as the presence of body hairs to which pollen sticks to. Many studies have reported that social Hymenoptera have greater capacity to transfer pollen than Lepidoptera or Diptera (Herrera 1987, Fishbein & Venable 1996). However, there are some cases in which this is not the case. Kumar *et al*. (1985) concluded that *Eristalis tenax* (Diptera: Syrphidae) can sometimes transfer greater amounts of pollen grains than *Apis* spp. Within the social Hymenoptera, *Apis mellifera* and *Bombus terrestris* are recognised as highly efficient pollinators, but again other authors have suggested that this depends on the crop in question. For example, *Megachile rotundata* (Megachilidae) is more efficient in alfalfa crops (*Medicago sativa*) than *Apis mellifera* (Bohart 1972). In other cases, some solitary Hymenoptera species have been found to carry greater amounts of pollen than *Apis mellifera* (Bosch & Blas 1994, Cane & Schiffhauer 2003).

Perhaps the most important confounding factor in determining the relative importance of pollinator species in gene flow is that total pollen load is not necessarily a good indicator of the quantity of viable pollen that is transported among flowers. Pollen viability is extremely variable, and depends heavily on the degree of hydration of the pollen prior to anthesis (Nepi & Pacini 1993), but it is typically highest in the first few hours immediately after flower opening. Even within the same flowers, Pylatuik *et al*. (1988) reported that pollen viability varied substantially depending on the position of the stamen. Consequently, the viability of pollen collected by flower visitors depends on where and when it is collected from flowers. Furthermore, several studies have shown that the viability of *Brassica* pollen carried by insects is negatively affected by adverse environmental conditions (Conner & Zangori 1997, Bots & Mariani 2005). On long distance foraging trips, pollen viability can decrease sharply (Nepi & Pacini 1993). In some pollinator species, the location of the pollen on the insect's body can also play a role in the rate of decline of pollen viability through time. For example, many bees place pollen in the pollen basket on the rear legs as an agglutinated pellet (Free 1970, Williams *et al*. 1994) and it has been found that pollen in these baskets has poor

viability because the glutination secretion reduces pollen viability, and the length of time that pollen is stored on the legs can be longer than the length of the pollen viability period of some plant species (B.J. Donovan, personal communication). By contrast, pollen on the body hairs of bees remains dry and viable for much longer (Bots & Mariani 2005). In some Lepidoptera, pollen is actually killed by a toxin secreted from the proboscis of the moth, which reduces the possibility of deposition of viable pollen on the stigma of the next flower visited (Richards *et al*. 2005). The relative viability of pollen transported by different insect species such as bees has rarely been investigated in the field (Bots & Mariani 2005). Also the results of some studies have not been suited for predicting the importance of pollen viability in transgene spread (Bots & Mariani 2005). Therefore, although many studies report large quantities of pollen transported by some pollinator species, this may not be a good indicator of pollination effectiveness and gene flow (Del Socorro & Gregg 2001).

Layout of the thesis

The broad aim of this thesis, then, was to examine how spatial and temporal variation in the abundance and behavioural activity of key insect pollinator species contributes to gene flow into and out of *Brassica rapa* crops. This thesis is made up of five chapters. Chapter One (this chapter) highlights the relevance of GM-crops and the risks of gene transfer to other cultivars or wild relatives. This chapter also describes the relevance of studying the behaviour of pollinators, and their importance in better understanding gene flow from crops. Chapter Two assesses variation in pollinator diversity and relative abundance during crop development, contrasting capture rates and directional movement inside and 50 m outside the crop border. Chapter Three evaluates species-specific variation in the behavioural activities of the five most important flower visitors to *Brassica rapa chinensis* throughout the crop flowering cycle. Chapter Four examines how total pollen loads and the viability of pollen carried by the five key pollinator species inside and outside the crop varied with flower phenology throughout the growing season. Finally, Chapter Five provides a discussion and synthesis of the risks of gene flow mediated by the behavioural activity of insect pollinators.

Chapter Two

Spatial and temporal variation in the relative abundance of key pollinators entering and leaving *Brassica rapa chinensis* crops throughout the flowering cycle: implications for gene flow in the landscape

INTRODUCTION

Cross-pollination is the main method of gene dispersal in flowering crops (Fenster 1991, Ellstrand & Elam 1993, Ennos 1994, Ghazoul 2005). Consequently, the abundance and diversity of the pollinator assemblage inside a crop can be very important for seed quality and production (Suberi & Sarker 1992, Stewart 2002). However, it is not only gene flow within the crop that is important to consider, but also gene 'escape' outside of the crop, which may result in the spread of engineered genes from genetically modified (GM) plant cultivars into conventional varieties (Poppy & Wilkinson 2005, Hoyle *et al*. 2007). Gene escape might be a particular problem when there is a wide range of different pollinator species with differing relative abundances and activity rates that might carry pollen (e.g., in *Brassica*; Abrol & Kapil 1996). Therefore, it is important to understand how the abundance and distribution of different pollinator species varies throughout the growing season (Howlett *et al.* 2005), and how the relative activity rates of different pollinator species entering and leaving the crop differs with changing crop phenology.

The rate of flower visitation by insects is likely to be an important determinant of reproductive success in crops (Sahli & Conner 2007). In *Brassica rapa* crops, cross pollination is dominated by insect pollinator activity (Hayter & Cresswell 2006). A large number of insect species visit *Brassica* flowers and they are thought to play a central role in the resulting quality and yield of seed (Bhalla *et al*. 1983). For example, some authors found that without adequate cross pollination *Brassica rapa* could not produce high seed yield (Williams & Simpkins 1989, Suberi & Sarker 1992, Westcott and Nelson, 2001). This coincides with Morandin & Winston (2005) who found that herbicide driven reductions in pollinator abundance resulted in poor seed set in GM *Brassica rapa* crops in Canada. It is likely that the effect of pollinator abundance on seed set is dependent on the cultivars planted, the environmental conditions where the crop grows, and the compensatory capacity of the crop (Williams *et al*. 1987, Mesquida *et al*. 1988, Free 1970, Westcott & Nelson 2001).

Brassica is visited by a wide range of pollinator taxa (Conner & Rush 1995), and there is a dramatic seasonal change in pollinator abundance with flower development (Williams 1985). Pollinator visitation can vary during the season because of environmental oscillations that lead to fluctuations in the population abundance of pollinator species (Herrera 1988, Fleming *et al.* 2001). Although there are a large number of *Brassica* visitors, only a small proportion of these visitors are responsible for the greatest proportion of plant reproduction (Lindsey 1984, Dewall & Thien 1989, Herrera 1989, Gomez & Zamora 1999). This is because crop visitors differ widely in their effectiveness for pollen transfer (Schemske & Horvitz 1984, Fishbein & Venable 1996). Morphological characteristics of pollinators such as tongue length and body size can also contribute to divergence in efficiency (Schemske & Horvitz 1984). Pollen transfer efficiency has also been reported to be influenced by the length of the visit period (Fishbein & Venable 1996, Ivey *et al*. 2003) and the extent to which visitation rate remains more or less constant over crop development (Sahli & Conner 2006). Sahli & Conner (2007) reported that in some cases the less efficient pollinator species in terms of pollen transportation were the most important pollinator for plant reproduction, simply because they were the most frequent visitors of *Brassica* crops.

The dominant taxa visiting *Brassica* flowers might also change seasonally according to the time when the seed is sown. Commercial fields of *Brassica* are sown twice a year, in autumn and in spring, flowering in spring and summer, respectively (Williams 1985). Experimental data in the U.K. showed that gene flow was twice as high in winter-sown crops, which bloom in May when the abundance of pollinators is scarce, than in spring-sown crops, which bloom in June when there are larger numbers of pollinators (Weekes *et al*. 2005).

The main pollinators which visit *Brassica* are Diptera, Lepidoptera, Coleoptera (Brunel *et al.* 1992, Chaudary 2001), solitary bees (*Leioproctus* and *Lasioglossum*) and social bees in the genera *Apis* and *Bombus* (Donovan 1980, Goodell & Thomson 2006). However, across all the taxa recorded as flower visitors of *Brassica* crops, *Bombus* spp. and *Apis mellifera* are the main visitors (Sihag 1986, Williams 1997, Singh & Singh 1992). These two species are considered to control the maximum rate of pollination (Heyter & Cresswell 2006). Therefore, variation in the abundance of these key pollinators, either spatially or seasonally may have a large impact on crop pollination (Firbank *et al.* 2003, Sahli & Conner 2007). The major role of bees might be explained because they deliver a hundred-fold greater amount of pollen than other pollinators (Degrandi- Hoffman *et al*. 1992, Hayter & Cresswell 2006). Also, bees may pass foreign pollen between bees in the nest (Ramsay *et al*. 1999), increasing the risk of gene flow from field to field. Ramsay *et al*. (2003) concluded that excluding bees from flowering crops may virtually eliminate gene dispersal. However, Hoyle *et al*. (2007) argued that the

modelled maximum level of bee-mediated adventitious GM presence in the seed of canola was significantly below the current European Union limit of 0.9%.

The aims of this study were to measure spatial patterns of abundance of key pollinator groups entering and leaving *Brassica rapa chinensis* crops, and to see how these spatial patterns varied through crop development in a late winter and a mid-spring planted crop.

MATERIALS AND METHODS

Field sites

Two 50 x 50 m fields of *Brassica rapa chinensis* (Pak-choi) were used in this study, and both were located at Lincoln on the Canterbury Plains, South Island, New Zealand. The soil in both fields is a Wakanui Silt Loam. *Brassica* seeds were drilled to 2 cm depth at 15 cm square spacing on 12 September 2006 (Lincoln 'Early season' field) and 10 November 2006 (Lincoln 'Late season' field). The fields were 3 km apart. The amount of seed sown was 200 kg seeds ha^{-1}. Weeds were controlled with Trifluran at 1.7 litres ha^{-1}. Fertilisation was applied according to common practices used in Crop and Food Research before planting (the details of rate of application and fertilizer composition are commercially sensitive and are not available for release).

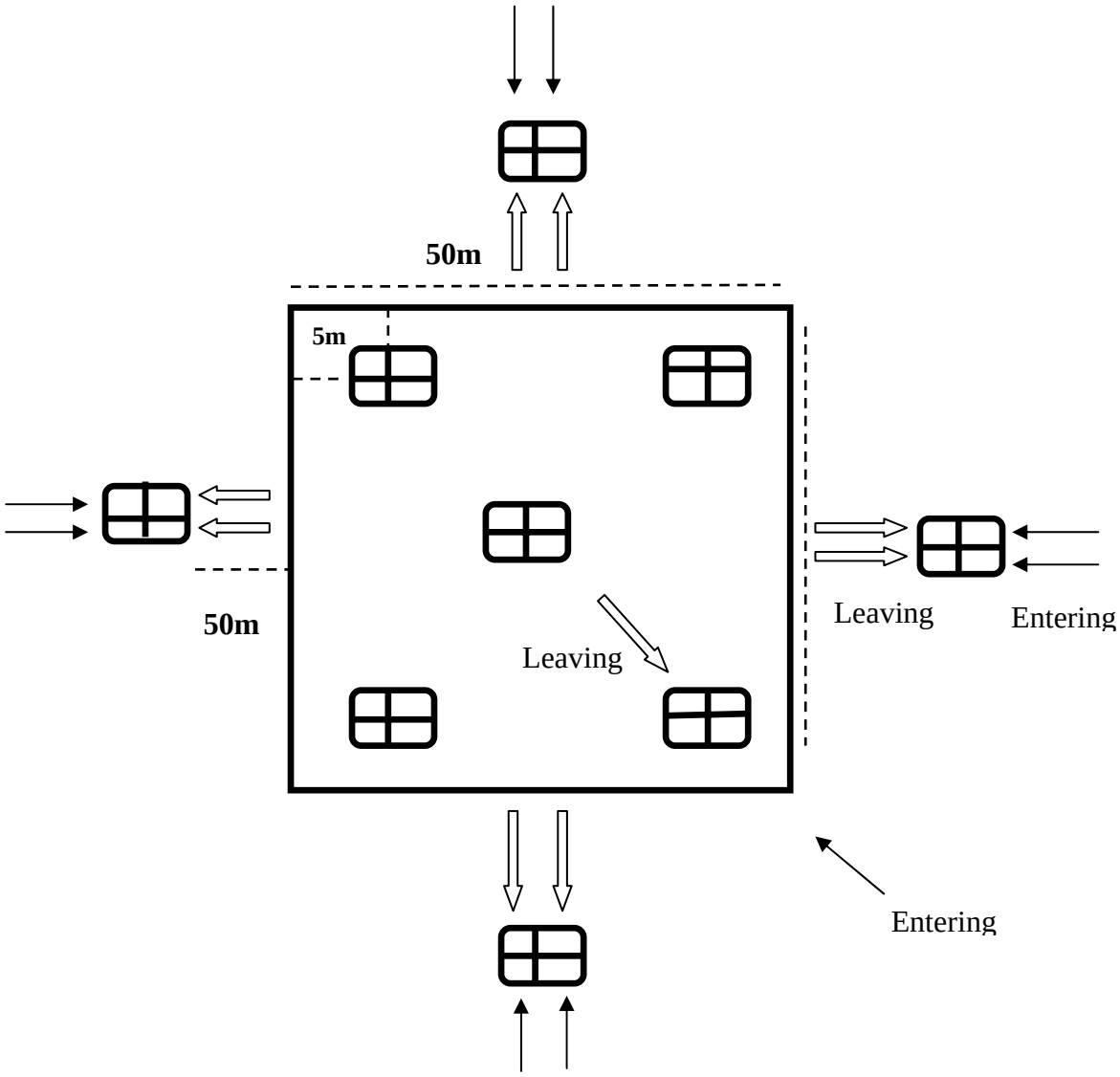


Figure 2.1. Diagram of a *Brassica* crop field showing the location of five window traps inside of the crop, one in the centre and four in the corners (5 m from the edge) with only one quadrant designated for the capture of insects "entering" the field, and the diagonally-opposite corner for the capture of insects "leaving" the field (in all four corner traps), and also the location of four window traps outside the crop (50 m from the edge of the field).

Crop development

Crop phenology was monitored at weekly intervals from the time the first leaves appeared on the emerging seedlings, until seeds were formed on mature plants, in both crops. On each sampling date, plant structural data were recorded within a 5-m radius area at the centre, and at each of the four corners of the crop, in the vicinity of a flight interception trap ('window trap') at each location. Three 1-m^2 quadrats were sampled at each of the five window trap locations inside the crop (giving a total of 15 samples per week), and plant and flower density

measurements were recorded. The number of plants in each 1-m^2 sample was recorded, and the number of inflorescences per plant was estimated by counting the inflorescences on 10 randomly selected plants. The numbers of buds, open flowers and old (senescent) flowers per inflorescence were estimated by counting the numbers of each flower type in 10 randomly selected inflorescences within each of the 10 plants. Plant height was measured with a measuring tape from the soil surface to the top of the tallest inflorescence on five plants in each quadrat. These variables were recorded weekly until the flowering phase had finished.

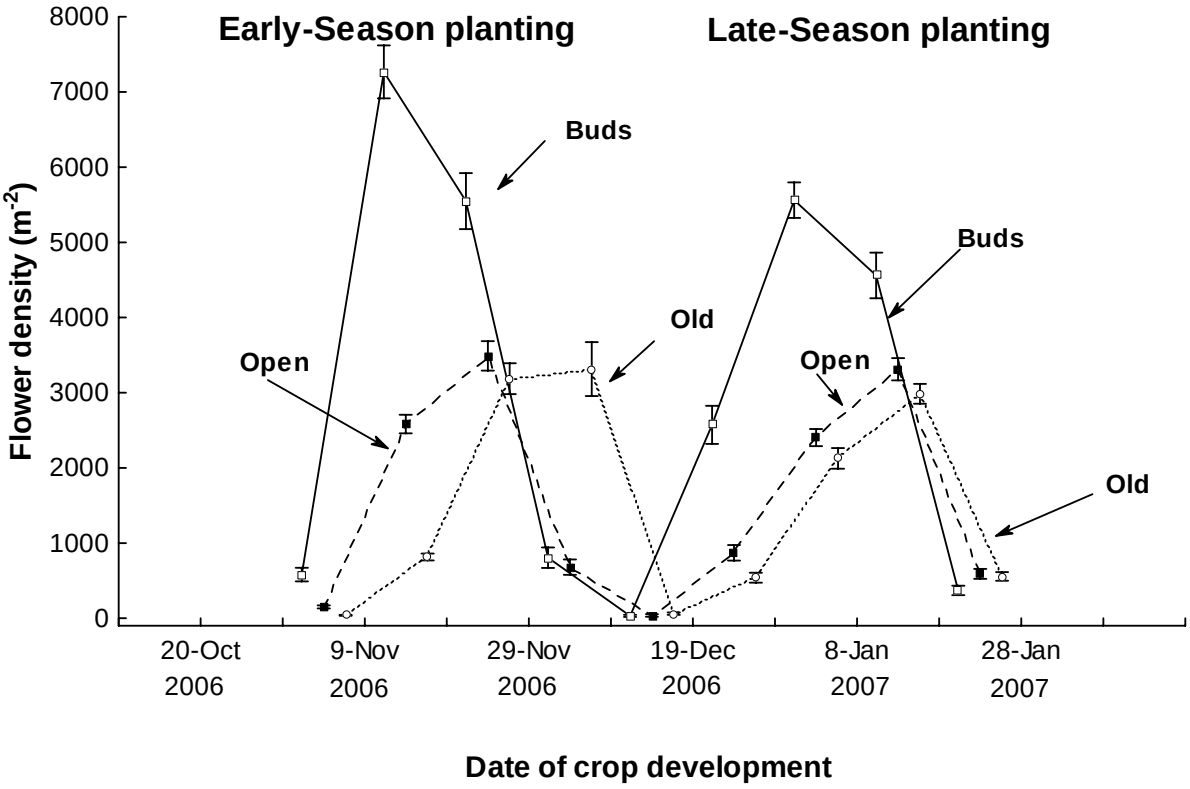


Figure 2.2. Variation in the densities of buds, open flowers and old senesced flowers during crop development, in both early-season and late-season plantings of *Brassica rapa chinensis* crops.

Pollinator diversity

Pollinator abundance was measured with four window traps (see Figure 2.1) located inside the crop, in each of the four corners (insect abundance data are not presented for the central trap). Additionally, four window traps were located outside the crop at 50 m distance from the crop edge. The window trap consisted of a grey 6 L plastic tray that supported a transparent Perspex window. The Perspex pane that ran lengthways along the tray had dimensions of 36.4 cm wide by 27.0 cm high (tapering at the tray base to 34.7 cm), while the pane running

perpendicular to this was 23.8 cm wide by 27.0 cm high (tapering to 21.8 cm wide at the tray base). Four long stakes (1.2 m aluminium coated with green plastic) were hammered into the ground at a height that was just below the height of the crop flowers, in a pattern that matched the trap dimensions. The grey plastic tray was attached to the stakes using 15 cm long copper tubing to connect the stakes with the plastic tray joiners. The window trap was then placed on top to ensure that it was positioned at the same height as the flowers. Window traps were oriented with the longest side pointing to the north. For the traps inside the crop (except centre traps), the exterior-most diagonal corner of each trap was designated as capturing insects "entering" the crop, while the interior-most diagonal corner of each trap was designated as capturing insects "leaving" the crop, as depicted in Fig. 2.1. Insect samples from the other two quadrants of each trap were not analysed here because it was much less likely that these could be interpreted as insects entering or leaving the crop with any great certainty. For the four traps outside the crop, the two quadrants of each trap that were closest to the crop were designated as capturing insects "leaving" the crop, and the two quadrants of each trap that were furthest away from the crop were designated as capturing insects "entering" the crop (Fig. 2.1). The collecting tray of each trap was filled with 1 L of water containing detergent. The detergent was used to reduce the surface tension of the water to ensure efficient capture of insects. The traps were left for five days, and then the insects were collected and placed in labelled vials containing 70 % ethanol. In the laboratory the capture rate of five key pollinators considered in the study was recorded each week. The five most common flower visiting species, *Bombus terrestris* (bumblebee, Hymenoptera: Apidae), *Apis mellifera* (honeybee, Hymenoptera: Apidae), *Lasioglossum sordidum* (Hymenoptera: Halictidae), *Eristalis tenax* (drone fly, Diptera: Syrphidae) and *Melangyna novae-zealandiae* (dark hover fly, Diptera: Syrphidae) were selected. Total pollinator abundance was defined as the absolute frequency of all five species of pollinators pooled together.

Figure 2.3. Window trap used for capturing key pollinators of *Brassica* flowers.

Statistical Analyses

Insect abundance is expressed in absolute and relative terms and used to describe relative importance of insect orders, families and species. These abundances are compared across groups, in early versus late season crops, over crop development, and with traps located inside and outside the fields. For comparisons of the abundance of pollinator insects entering and leaving fields, only two quadrants within an individual trap inside the field could be designated as ‘entering’ or ‘leaving’ with a great deal of certainty (see above). Therefore, because counts from traps inside the crop were only made from two of the four trap quadrats (in each of the four traps inside the field), whereas counts from traps outside the crop were made from all four trap quadrats of each of the four traps outside the field, it was necessary to standardize abundance per trap by multiplying counts by a factor of two for all traps inside the field. Contingency tables (2×2) were calculated to determine whether the frequency of insects entering versus leaving the field differed between traps inside and outside the field using Chi-square tests.

RESULTS

Window trap survey

Six orders from the class Insecta were captured in window traps over the flowering period, with a total of 10,061 and 6,654 specimens from the early season and the late season fields, respectively. Diptera was the order with the highest number of specimens (12,823) and species captured (18) belonging to 10 different families (Appendix 2.1). Diptera abundance was greater in the early season crop (8,213) than in the late season crop (4,610), and was typically greater inside than outside the crop in both cases (Figure 2.4). Hymenoptera was the second most abundant order with 2,872 specimens and 7 species, with the largest number of specimens represented by *Apis mellifera* (1,016 specimens), *Lasioglossum sordidum* (969 specimens) and *Bombus terrestris* (552 specimens) (Appendix 2.1). Unlike Diptera, Hymenoptera had a greater abundance in the late season crop than in early season crop, and Hymenoptera abundance was generally lower outside the crop than inside, with one notable exception at the peak of flowering in the late-season planting (Figure 2.4).

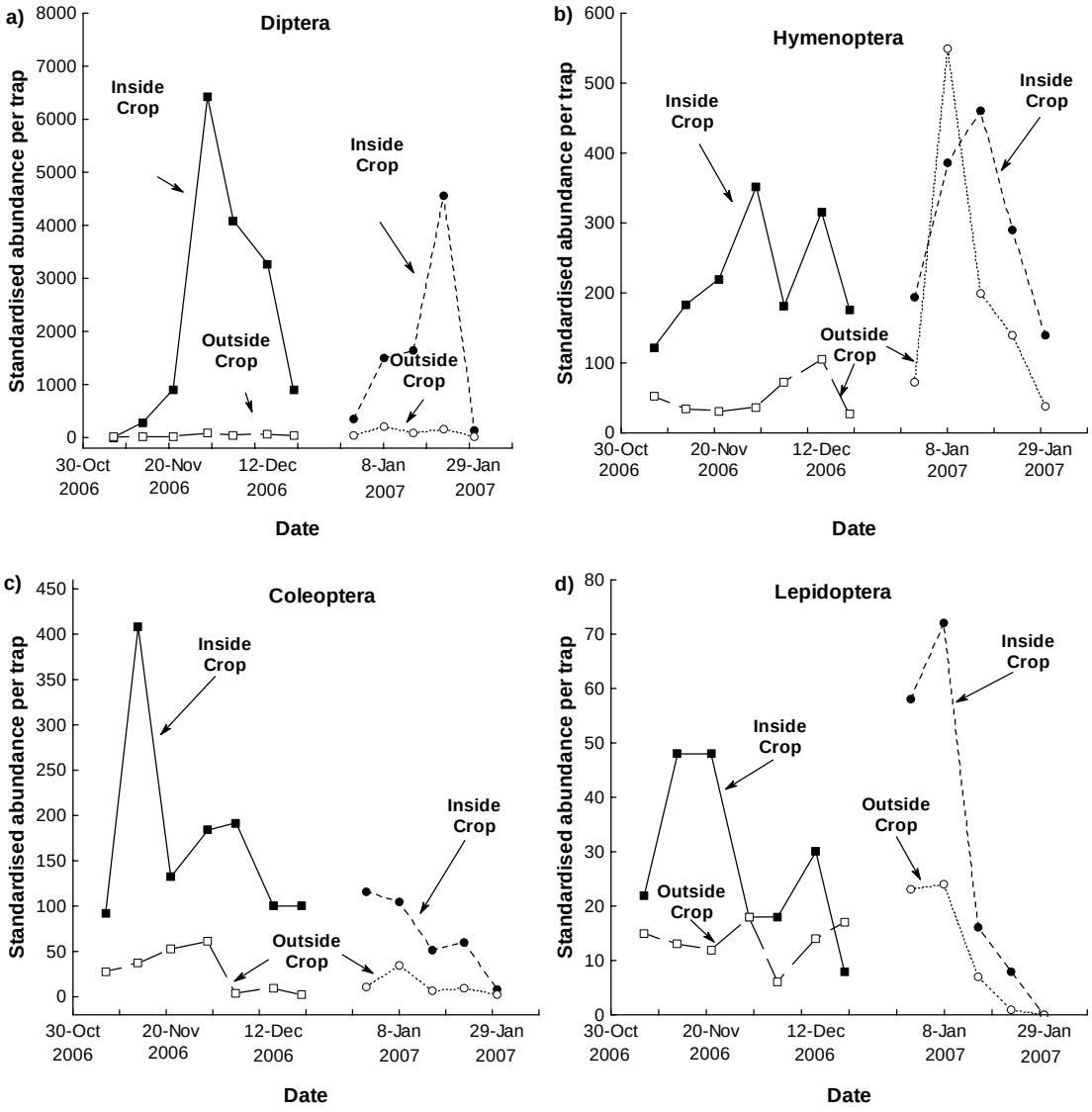


Figure 2.4. The four most abundant insect orders captured in window traps located inside and outside *Brassica rapa chinensis* crops in both early- and late-season plantings: a) Diptera, b) Hymenoptera, c) Coleoptera and d) Lepidoptera. Counts from traps inside the crop were only made from two of the four trap quadrants (in each of the four traps inside the field), whereas counts from traps outside the crop were made from all four trap quadrants of each of the four traps outside the field (see Methods). Therefore, standardized abundance per trap was calculated by multiplying counts by 2 for all traps inside the field.

Twenty-one families from six orders in the class Insecta were captured in window traps over the flowering period (Figure 2.5). The most frequently captured family was Stratiomyidae (Diptera) with 7,241 specimens captured in early season and 3,727 in late season. The most common families from Hymenoptera were Apidae (856 specimens in early season and 712 in late season) and Colletidae (269 specimens in early season and 1,274 in late

season). The dominant family from Coleoptera was Scarabaeidae with 484 specimens in early season but only 4 in late season.

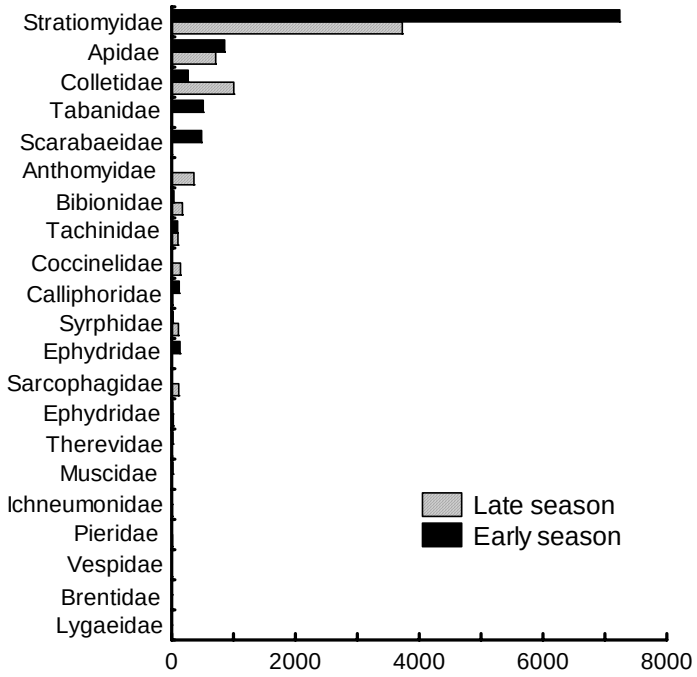
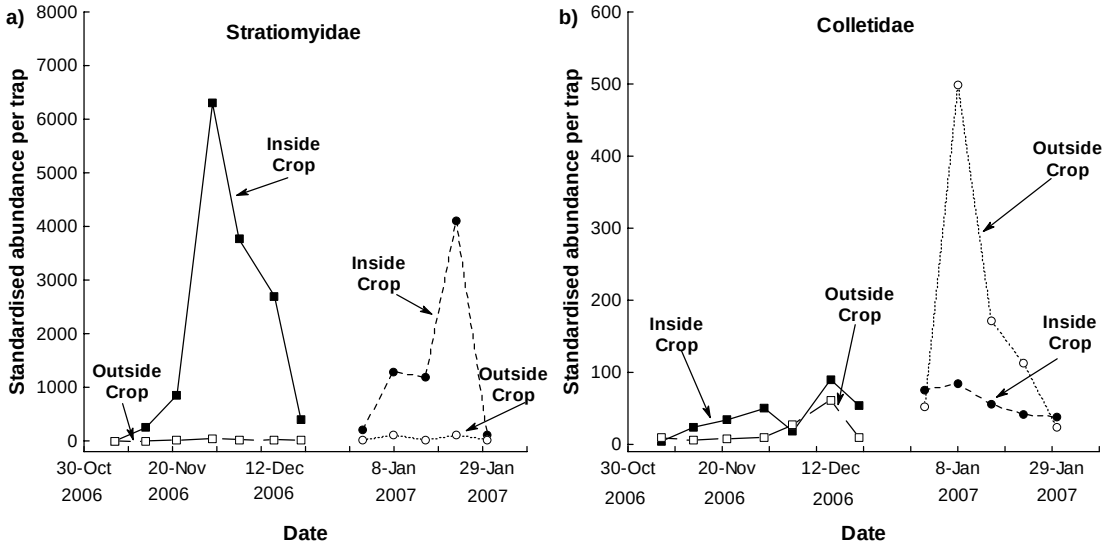


Figure 2.5. The relative abundance of insect families captured in early- and late-season plantings of *Brassica rapa chinensis* (traps inside and outside the crop combined).

Figure 2.6 shows capture rates for key families inside and outside the early- and late-season crops. General trends for the most abundant families show that: (a) capture rates follow the crop phenology i.e. greatest rates at peak of flowering, (b) greater capture rates in early than late season crops, and (c) greater captures rates inside than outside the crop.



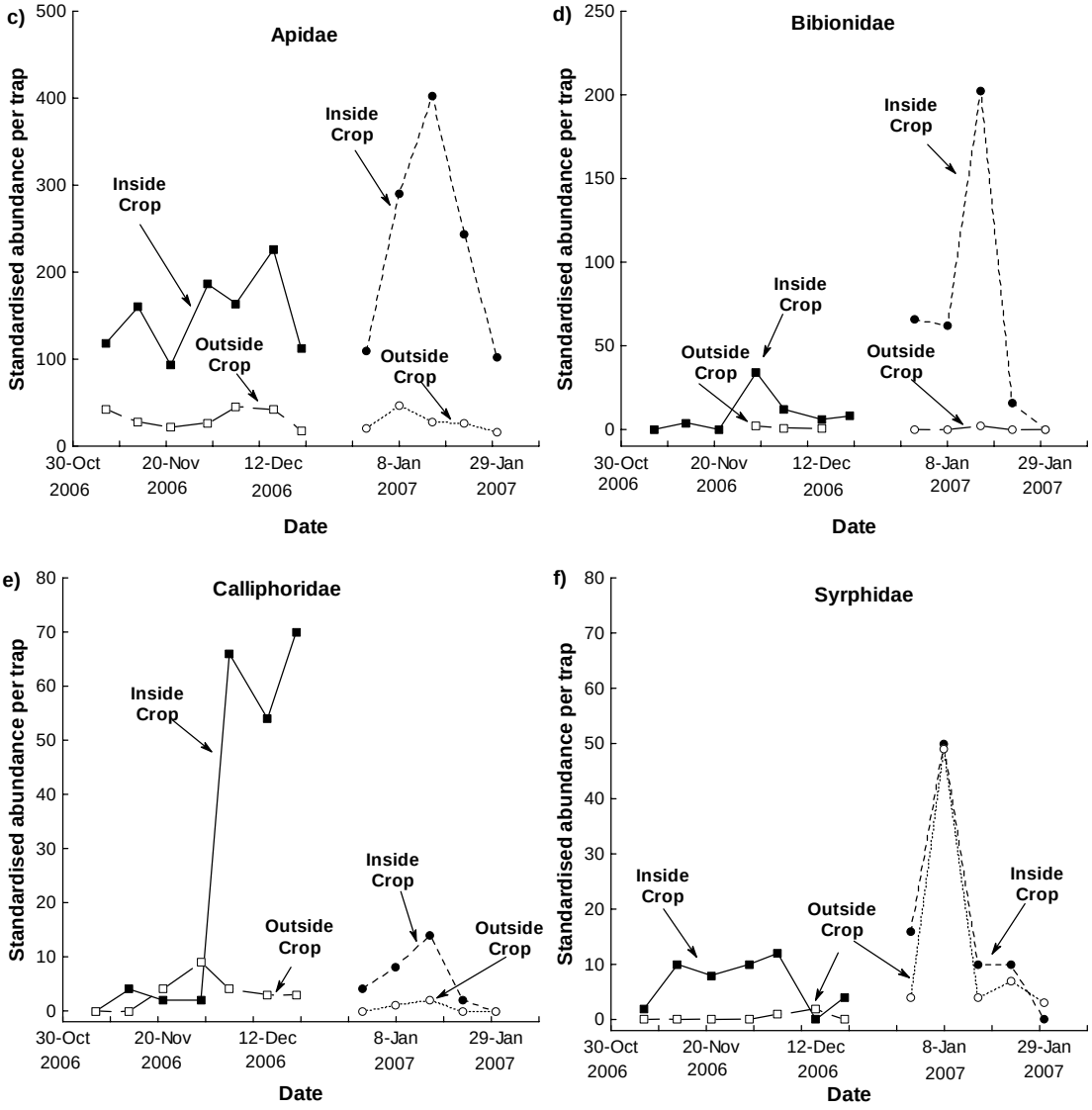
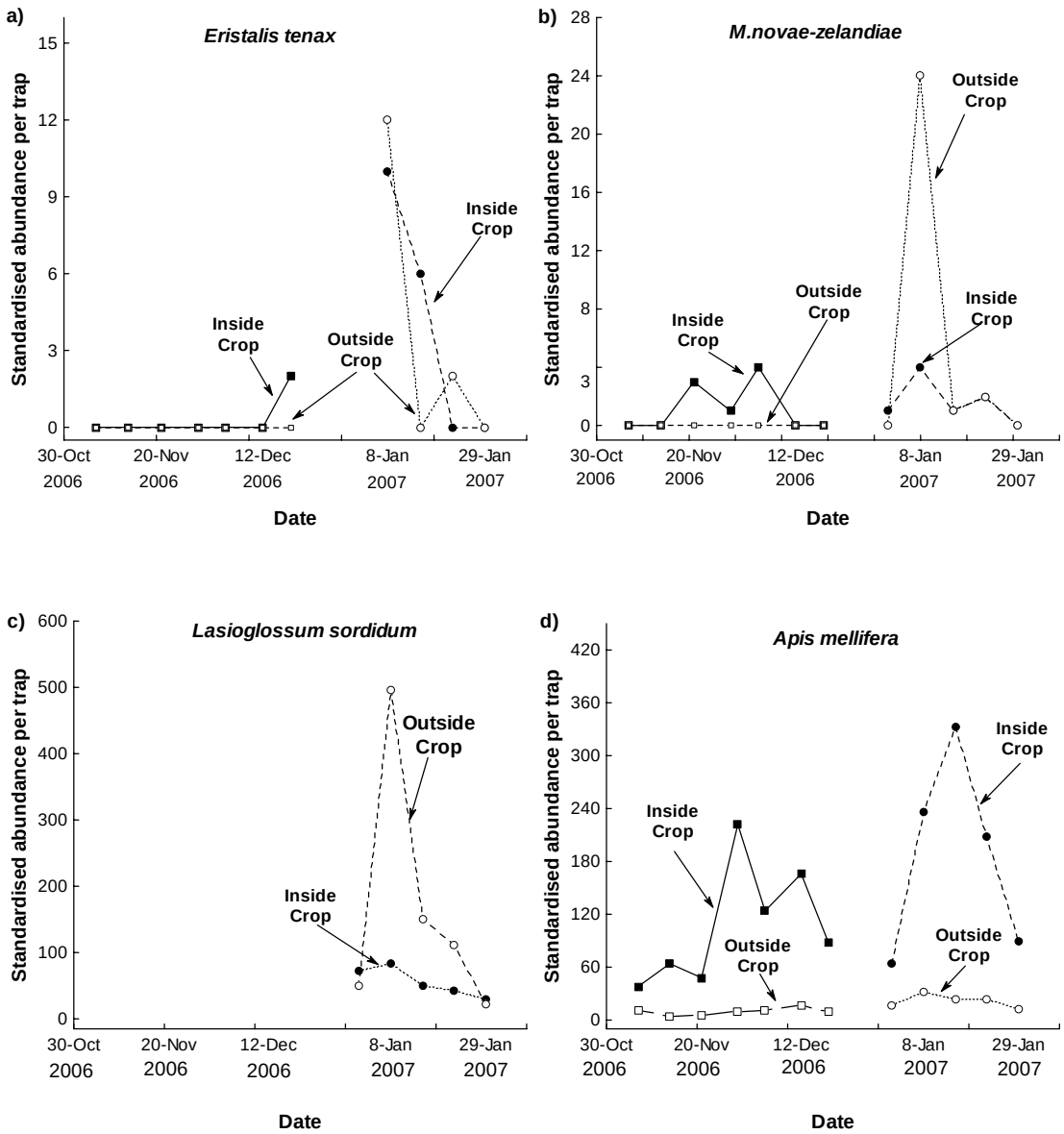


Figure 2.6 The most abundant insect families captured in window traps located inside and outside *Brassica rapa chinensis* crops in both early- and late-season plantings: a) Stratiomyidae, b) Colletidae, c) Apidae, d) Bibionidae, e) Calliphoridae and f) Syrphidae. Counts from traps inside the crop were only made from two of the four trap quadrants (in each of the four traps inside the field), whereas counts from traps outside the crop were made from all four trap quadrants of each of the four traps outside the field (see Methods). Therefore, standardized abundance per trap was calculated by multiplying counts by 2 for all traps inside the field.

Five species identified as key pollinators in previous studies accounted for 2,603 specimens, dominated by *Apis mellifera* (39%), *Lasioglossum sordidum* (37%) and *Bombus terrestris* (21%), while *Eristalix tenax* (1%) and *Melangyna novae-zealandiae* (2%) were comparatively less abundant. *Apis mellifera*, the most abundant key pollinator, showed a consistent pattern of abundance that resembled crop phenology for both the early and the late

season crops (Figure 2.8). *Apis mellifera* abundance was also consistently greater inside than outside the crop. The pattern observed for *Bombus terrestris* coincided with the one observed for *Apis mellifera* (Figure 2.8). *Lasioglossum sordidum* was only present in late season, and showed a pattern that also resembled flower development. However, in contrast to *Apis mellifera* and *Bombus terrestris*, absolute frequency of *Lasioglossum sordidum* outside the crop was substantially greater than inside the crop. *Eristalis tenax* and *Melangyna novae-zealandiae* were comparatively rarely captured and therefore trends in relative abundance were not possible to establish.



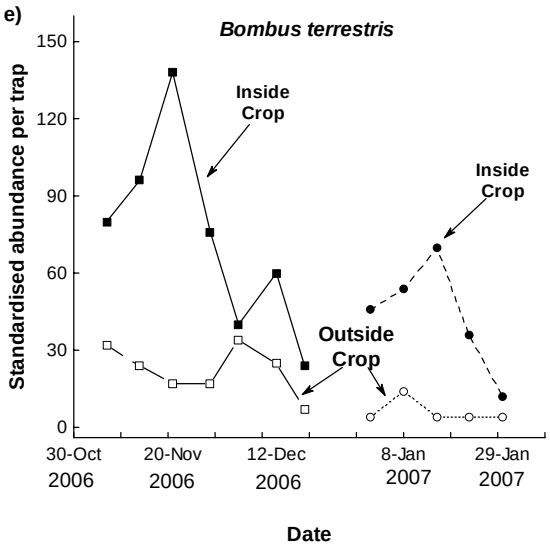


Figure 2.8 The five key pollinators captured in window traps located inside and outside *Brassica rapa chinensis* crops in both early- and late-season plantings: a) *L. sordidum*, b) *A. mellifera*, c) *B. terrestris*, d) *M. novae-zelandiae* and e) *E. tenax*. Counts from traps inside the crop were only made from two of the four trap quadrants (in each of the four traps inside the field), whereas counts from traps outside the crop were made from all four trap quadrants of each of the four traps outside the field (see Methods). Therefore, standardized abundance per trap was calculated by multiplying counts by 2 for all traps inside the field.

The overall frequency of insects entering versus leaving the field differed significantly between traps inside and outside the field for the late season crop (χ^2 =7.050, P = 0.008), although not for the early season crop (χ^2 = 2.050, P = 0.152). Two species in the early season crop (*Megathereva bilineata* and *Scaptia adrel*) and seven species in the late season crop (Tachinidae spp., *Delia platura*, *Eristalis tenax*, *Helophilus hochstetteri*, *Leioproctus* sp., Lepidoptera Moths and *Pieris rapae*) differed significantly (all χ^2 > 4.29, P < 0.038) in the frequency of insects entering versus leaving between traps inside and outside the crop (Appendix 2.1).

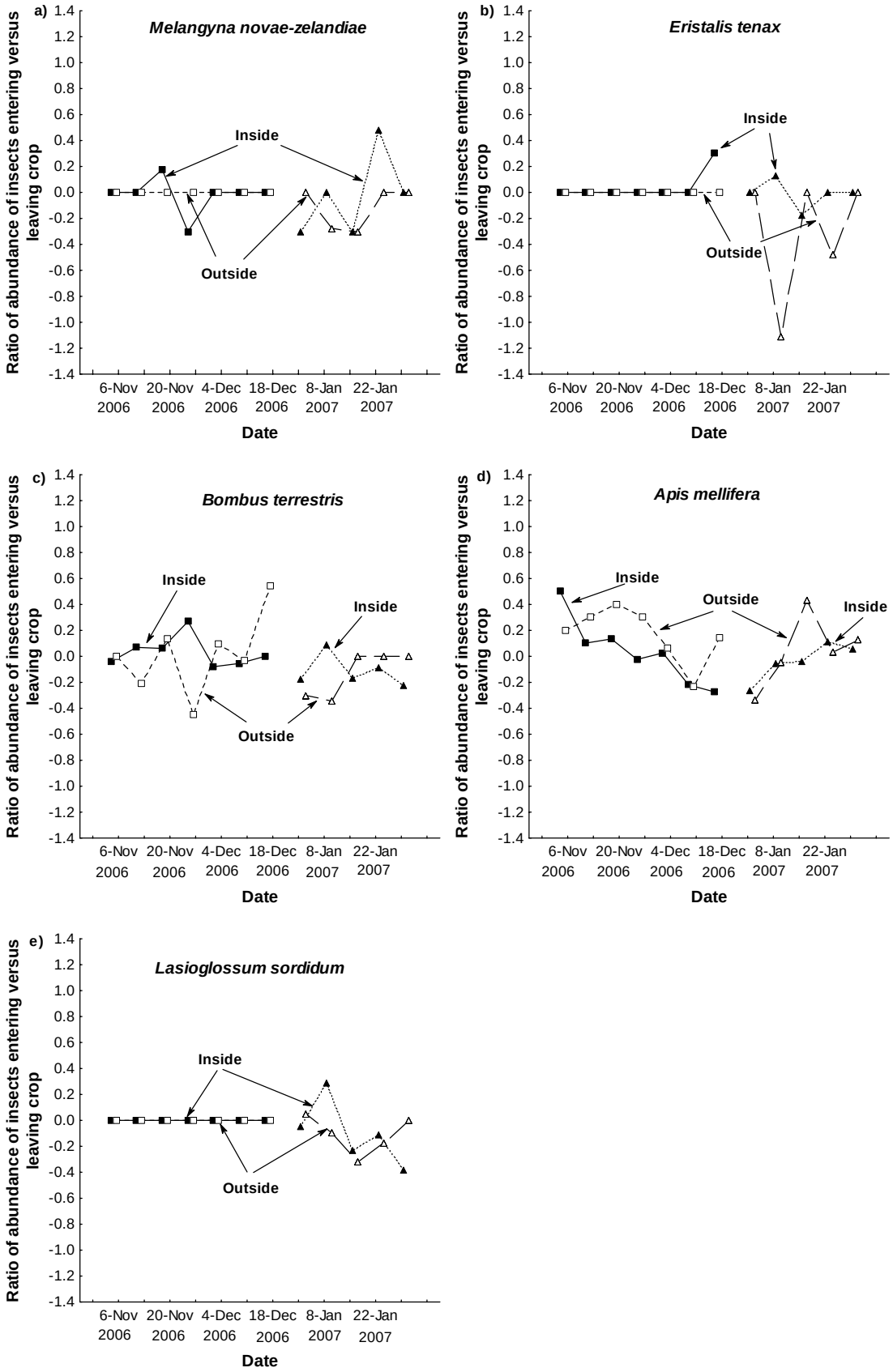


Figure 2.9 Ratio of abundance of five key pollinator species captured entering versus leaving *Brassica rapa chinesis* crops using window traps located both inside and outside the crop, in early-season and late-season plantings.

The log ratios of insects entering versus leaving varied considerably within seasons (Figure 2.9). Ratios of *B. terrestris* varied the most among key pollinators from -0.44 to + 0.54, and progressively less in *M. novae-zelandiae* (-0.30 to + 0.47), *L. sordidum* (-0.39 to +0.29) and *E. tenax* (-0.18 to + 0.12). With such wide variations, trends were difficult to find. However, log ratios of *A. mellifera* (early crop) and *L. sordidum* (late crop) varied from positive early in the crop to negative late in the crop, showing that there is a switch from more individuals entering to more leaving as the crop ages. However this was not always the case. For instance, log ratios of *A. mellifera* (late crop) and *M. Novae-zelandiae* (early crop) tended to be negative early in the crop and positive late in the crop.

Discussion

Flower visitors play a vital role in the pollination of many flowering crops (Free 1970, Westerkampe & Gottsberger 2000). However, flower visitors may also contribute to undesirable gene escape from crops, including genetically-modified cultivars (Funk *at al*. 2006). Hence, it is essential to understand how the diversity and spatial distribution of flower visitors varies during crop development. I found similar diversity at the family level for both crops studied, although capture rates were greater in the early than in the late season crop for most species. However there were some species, noticeably *Lasioglossum sordidum* and *Eristalis tenax*, that only appeared during the late season crop. This might be related, as suggested by Donovan (1980), to more favourable environmental conditions, such as air and soil temperatures, in the late season crop.

I observed an increase in the number of insects captured during the peak of flowering. This has also been observed in oilseed rape, where mass flowering has been associated with high insect abundance (Westphal *et al*. 2003). This increase cannot be entirely attributed to insects emerging from their larval stages within the field, but instead to insects flying in from their nearby nesting or hibernating habitats. Therefore insect abundance and diversity are likely to be influenced by the local and landscape distribution of flowering plants.

Diptera, Hymenoptera, Coleoptera and Lepidoptera were the most abundant orders found in this study. Sahli & Conner (2007) found similar results in a study in another Brassicacea crop i.e. *Raphanus raphanistrum*. Diptera was the most abundant order, and Stratiomyidae was the most abundant family of Diptera collected. Diptera was more abundant in the early than in the late season crop, and population abundance is thought to be determined by warm temperatures > 12.8 °C (Roberton 1992). Little is known of the pollination ability of Stratiomyidae, although it has been recognized as a flower visitor and hence a potential agent of pollen transfer in *Brassica* crops (Lamborn & Ollerton 2000, Souza-Silva 2001). Even if

Stratiomyidae are relatively ineffective pollinators, their great abundance would increase their relative role in pollen flow through crops. However, more research is needed to know whether they are likely to transfer pollen from one crop to another.

Syrphidae represented only a small proportion of Diptera, and smaller numbers of this family were captured in the early than in the late season crop. Some authors have reported that Syrphidae are attracted to Brassicaceae and that they carry substantial amounts of pollen on their legs and hairy body (Holloway 1976, Conner *et al*. 1995). Although syrphid flies (*Melangyna novae-zelandiae* and *Eristalis tenax*) do not have specific morphological adaptations for pollination, they might contribute to transfer pollen from oilseed rape to wild *Brassica* species contributing to gene flow (Strauss *et al*. 1999). Some syrphid species are also known to move up to several kilometres between crops (Golding *et al*. 2001).

The second most abundant group of flower visitors in this study were from Hymenoptera. This is similar to the findings of Hayter & Creswell (2006), who concluded that Hymenoptera are highly efficient in the pollination of *Brassica*. The most abundant families in Hymenoptera were Apidae (*Apis mellifera*) and Halictidae (*Lasioglosum sordidum*). Pierre *et al*. (2003) also found these families to be the most abundant Hymenopera in *Brassica*. I found Hymenoptera to be more abundant in the late-season crop than in the early-season crop. Several authors (Herrera 1989, Fleming *et al*. 2001, Hayter & Creswell 2006, Sahli & Conner 2007) have found similar results, with bees varying greatly both seasonally and spatially in *Brassica napus* fields.

Among the five key pollinators selected for this study *Apis mellifera* was the most abundant. Other authors have found the same (Bhalla *et al*. 1983, Bosh *et al*. 1997, Fishbein &Venable 1996), and not surprisingly the abundance of *A. mellifera* seems to be heavily influenced by the distance of oilseed rape fields from commercial beehives (Sabbahi *et al*. 2005). In my study, the abundance of *Apis mellifera* tracked crop development in both fields, with greater numbers of individuals captured inside than outside the field. This might be expected as massive flowering odour and colour attracts pollinators (Westphal *et al*. 2003).

Lasioglossum sordidum was found in the late season crop only. I attribute this phenomenon to insects emerging from their larval stages at this time and not before (Donovan 1980). Solitary wild bees are being progressively recognized as important pollinators within flowering crops (Kremen *et al*. 2002, Fontaine *et al*. 2006, Hayter & Cresswell 2006), and solitary bees have a high potential to transfer pollen from oilseed rape to wild relatives.

I found *Bombus terrestris* to have slightly lower abundance than *Apis mellifera. Bombus terrestris* is well known for its pollinator capacity (Free 1970, Creswell 1999, Creswell 2004) being very common in *Brassica* crops (Carvell 2007). *Bombus* species have the ability to

carry pollen on hairs on the body for long distances, so they might be an important agent of gene flow (Osborne 1999).

E. tenax and *M. novae-zelandiae* were infrequently captured and did not show a clear pattern of abundance over crop development. *M. novae-zelandiae* has been recognized as a *Brassica* visitor and long-distance disperser species hence contributing to gene escape from crops (Silberbauer 20004). On the other hand, Jarlan (1997) reported that *E. tenax* has desirable traits for pollination of sweet pepper, carrot (Umehara *et al*. 2005) and *Brassica* (Adegas 1992).

There was no clear overall seasonal change in the frequency of insects entering versus leaving the crop despite that crop phenology changed dramatically. However, it is worth noting that not uncommonly more insects were attracted into the crop early in the season, staying there rather than leaving (a positive log ratio), and then when flowers started to disappear there was a large peak in insects leaving (a negative log ratio). This phenomenon was also observed for *A. mellifera* by Meier (2007). We might speculate that pollinator responses to massive changes in flower development might be modest explaining the lack of a strong response for all key pollinators studied.

Capture rates were much greater inside the crop than 50 m from its border for both the early- and the late season crop. One reason for this is that *Brassica* crops are known to attract pollinators by the colour and the smell of the flowers (Stephenson 1982, Hoyle *et al*. 2007). Also, Chittka & Thomson (2001) argue that pollinators are able to memorize resource locations where nectar and pollen in a particular flower patch are in abundance, reaching these spots in the following foraging trips being this phenomenon called constancy. Therefore there must be a synchrony between pollinator abundance and flower development inside the field (Légère 2005), as opposite to outside the crop where only grass was available.

Differences in capture rates throughout the season showed that the major risk of gene flow occurred at the peak of flowering. This is because the greatest amount of pollinators were attracted at this time, and not uncommonly more insects left that entered after the peak of flowering. A large number of pollinators leaving the crop may carry large amounts of pollen outside the crops where cross-pollination with wild relatives might occur. That is coincident with Heinrich (1975) who reported that outcrossing occurs predominantly during the peak of blooming.

In summary, spatial patterns of abundance of key pollinator groups entering and leaving *Brassica rapa chinensis* crops were measured in a late winter and a mid-spring planted crop, in order to see how these spatial patterns varied through crop development. I found an increase in the number of insects captured during the peak of flowering, far greater capture

rates inside than outside the crop, and a not uncommonly trend of more insects entering than leaving early in the crop switching progressively to the opposite late in the crop.

Chapter Three

The influence of crop phenology on insect pollinator behaviour in a *Brassica rapa chinensis* crop

INTRODUCTION

In flowering plants, pollination is the main mechanism of gene dispersal (Fenster 1991, Ellstrand & Elam 1993, Ennos 1994, Ghazoul 2005). Gene dispersal through cross-pollination depends on pollen transport by external agents (Lloyd 1992), and insects are amongst the most important pollinator agents for many plant species (Free 1970). The specificity of insect mediated pollination and the nature of insect foraging behaviour have an important influence on patterns of gene flow (Schmitt 1980, Waser 1988). Although much is known about the general influence of microclimatic factors such as temperature, relative humidity and solar radiation on pollinator behaviour (Vicens & Bosch 2000, Kumar & Singh 2003), behavioural responses are highly species-specific and there is relatively little information on temporal variation in relative species responses to changing floral phenology throughout the season. For instance, *Apis mellifera* (Hymenoptera: Apidae) becomes active only at temperatures higher than 12-14 °C and solar radiation greater than 300 Wm^{-2} (Vicens & Bosch 2000). There also seems to be an upper microclimatic threshold over which foraging activity decreases, but again these responses are species-specific. For instance, *Bombus* spp. and *Apis* spp. showed reduced foraging activity at irradiance over 1100 W m^{-2}, whereas flies (Diptera) reduced activity due to overheating at only 600 W m^{-2} (Herrera 1990, Collins *et al.* 1997). Foraging rates have been shown to be positively correlated with relative humidity for *B. terrestris* (Peat & Goulson 2005), while for some other species (e.g. the wasp *Parachartergus fraternus*) foraging rates have shown a negative correlation (Paula *et al.* 2003). Finally, wind speed is another factor influencing pollinator behaviour. Strong winds have been reported to reduce foraging distance and activity of bees, because wind makes foraging and high flight speed difficult (Kevan & Baker 1983, Totlan 1994, Osborne *et al.* 1999, Vicens & Bosch 2000).

Understanding the role of insect pollinator behaviour in gene flow between plants also requires detailed knowledge of variability, or plasticity, in behavioural response to changing plant structure and flowering phenology throughout the growing season. This is particularly important in intensively cultivated cropping situations, where dramatic seasonal changes in flower density and the flowering phenology of the crop can have important effects on the pattern of movement of pollinators (Schmitt 1983, Handel & Mishkin 1984), compared to

behavioural activity in areas of sparse flower availability. For example, in high density flowering crops, gene flow at peak flowering can extend much further than any single pollinator movement; because pollinators can transfer pollen in several flower-to-flower steps, known as pollen carry-over (Levin 1981, Schmitt 1983). For these reasons, several authors have concluded that the dynamics of pollination in flowering crops will remain poorly understood until future research provides a more complete picture of the insect behavioural responses at individual flowers and seasonal changes in behaviour with changing crop phenology (Eisikowitch 1981, Handel & Mishikin 1984)

The goal of this study was to investigate species-specific variation in the behavioural activity of multiple pollinator species with changing flowering phenology in a cultivated *Brassica rapa chinensis* crop. Several *Brassica* species are common weeds and important agricultural crops in Europe and North America and *Brassica rapa* is the second most important crop in Canada, after wheat (Mohr & Jay 1990, Conner & Zangori 1997). The Canterbury Region of New Zealand produces many seed crops of *Brassica* for oil-seed and vegetable production (Stewart 2002).

Brassica rapa is an excellent research crop to study patterns of variation in pollinator behaviour because it has a rapid life cycle, does not self pollinate and require insect or wind for cross pollination (Stewart 2002). The structure of *Brassica* flowers is well adapted to generalist insect pollinators, it has colourful petals, large amounts of pollen, scent production and continues nectar production during the whole flowering period, which attracts insects to feed (Free 1970).

Diptera and Hymenoptera are regarded as the most important pollinators of *Brassica* species (Brunel *et al*. 1989, Brunel *et al*. 1992, Chaudhary 2001, Easthan & Sweet 2002). Previous studies have shown that there is strong species-specific variation in pollinator foraging behaviour in *Brassica rapa* crops. For instance, *A. mellifera* is thought to be the most efficient pollinator in *Brassica rapa* crops because they carry pollen not only on their legs but also on the body setae (Free 1970, Eastham & Sweet 2002). The behaviour of *Apis mellifera* tends to increase cross-pollination rates, because bees have a tendency to move between clusters of flowers on different plants, rather than staying in one cluster on a single plant (Langridge & Goodman 1975). Honey bees also deliver pollen to flowers more quickly than other pollinators (Hayter & Cresswell 2006). In one study, Ramsay *et al*. (1999) further suggested that mixed-source pollen is passed between bees in the nest increasing gene flow and cross-pollination on subsequent foraging trips. However, Hoyle *et al*. (2007) stated that honey bees and bumblebees are actually only responsible for relatively short-distance gene

flow, most likely because pollen from unrelated plants is deposited on the first flowers visited and is not carried across many flowers (Cresswell *et al.* 2002).

In addition to honeybees, bumblebees of the genus *Bombus* are also one of the main pollinators recognised on *Brassica rapa* crops (Goodell & Thomsom 2006). Creswell (1999) reported that bumblebees can deliver 150 grains of pollen to the stigma of a flower during a single visit. Furthermore, Hayter & Cresswell (2006) reported that bumblebees can be more common flower visitors in *Brassica* crops than honeybees, and that bumblebees forage twice as fast as honey bees in *Brassica* flowers. Westerbergh & Saura (1994) added that bumble bees are very active in their movement between plants within a patch of flowers, and also moved to a distant patch, thus increasing the possibility of gene flow. Bumblebees make longer trips because they are less restricted by nest site requirements and they can benefit from mass flowering crops such as *Brassica* (Westphal *et al.* 2003), and because bumblebees have a higher thermoregulatory capacity and they can be more active compared to other bees, in cool conditions (Heinrich 1975b).

Finally, among the Hymenoptera pollinators, solitary bees are also cited as important *Brassica* visitors in some cases (Bhalla *et al.* 1983). Although Langridge & Goodman (1975) reported that populations of solitary bees visiting *Brassica* crops are not large enough to ensure pollination, Morandin *et al*. (2007) reported that the reason for low densities of solitary bees is because they nest in the ground and *Brassica* fields are often tilled twice a year, destroying the nests of wild bees. Raw (2000) concluded that small solitary bees pollinate flowers of *Capsicum annuum* effectively when plants are grown relatively close, because they have a smaller foraging area. However, other research by Raw (1976) has shown that some larger solitary bees are capable of bearing larger pollen loads over longer distances than smaller bees can do, and in general the capacity to make long trips depends on the size of the bee (Waddington *et al.* 1994, Steffan-Dewenter & Tscharntke 1999). In fact, some solitary bees can reach nearly 1 km on their foraging trips (Steffan-Dewenter & Tscharntke *et al.* 1999, Gathmann & Tscharntke 2002, Morandin & Winston 2005). Unfortunately, the contribution of wild solitary bees to crop pollination is still unclear in most instances (Kearns & Inouye 1997, Kevan & Phillips 2001). Morandin & Winston (2005) demonstrated that crop yield in *Brassica* increases with increasing abundance of wild bees. In New Zealand, the most common solitary native bees pollinating onion crops and Brassicaceae crops are from the genera *Lasioglossum* (Halictidae) and *Leioproctus* (Colletidae) (Howlett 2005) with important contributions to seed set in these crops.

In addition to Hymenoptera pollinators, a number of important Diptera pollinators have been recorded on *Brassica* crops. Perhaps the most important Diptera pollinators of

Brassica are hover flies (Syrphidae) (Conner & Rush 1995). Syrphids certainly do consume pollen (Conner & Rush 1995), but they also transfer a large amount of pollen on their body to other flowers (Herrera 1987). When Syrphid pollinators seek nectar their heads become powdered with pollen and they can act as excellent agents of pollen dispersal (Westerbergh & Saura 1994). In some instances, though, Rush *et al.* (1995) noted that Syrphids may only carry large pollen loads from the first visitation to a flower, because the body of the insect becomes saturated in the following flower visits. Generally speaking, then, hover flies are often considered to be less effective pollinators than bees, but they nevertheless play an important role in cross-pollination (Hoyle *et al*. 2007). Another important characteristic of hoverflies is that they have a seasonal behaviour that allows them to contribute differentially to gene flow at different times during the flowering cycle (Langridge & Goodman 1975). Hoyle *et al*. (2007) reported that Syrphids visited fewer plants in succession than bumblebees, but tended to visit a few adjacent plants and then suddenly fly several metres away and resume small-scale flower visitation once again, thus increasing cross pollination and gene flow (Westerbergh & Saura 1994).

Some early studies initially suggested that blow flies (Calliphoridae) tend to rest in flowers, but do not seek nectar or pollen, and thus do not have a role in cross-pollination (e.g., Langridge & Goodman 1975). However, other authors have reported blow flies as active pollinators of *Brassica* crops (e.g., Mesquida *et al*. 1988), and Currah & Ockendon (1983) concluded that blowflies of the genus *Calliphora* were as effective in cross pollination as bees. Later research in Scandinavia also reported that although blow flies carry small pollen loads they are important pollinators (Westerbergh & Saura 1994). In New Zealand, several species in the genus *Calliphora* are common and research has shown that there are significant seasonal trends in their abundance (Currah & Ockendon 1984).

Species-specific variation in the behavioural activities of different pollinators throughout the crop flowering cycle are therefore crucial to a comprehensive understanding of the processes that control gene flow within and between intensively-cultivated crops. Policy makers are particularly concerned about transgenic introduction into non-GM crops of the same species in the vicinity of a GM crop and the introduction of a transgene via hybridization to wild relatives of the crops via pollen carried by insects (Weekes *et al.* 2005). In order to avoid cross-pollination there are recommended minimum isolation distances between GM crop and conventional ones (SCIMAC 1999).

In this study, I tested the influence that crop age has on flower visitor behaviour for the five most important flower visitors to *Brassica rapa chinensis* in Canterbury, New Zealand, which were *Bombus terrestris* (bumblebee, Hymenoptera: Apidae), *Apis mellifera*

(honeybee, Hymenoptera: Apidae), *Lasioglossum sordidum* (Hymenoptera: Halictidae), *Eristalis tenax* (drone fly, Diptera: Syrphidae) and *Melangyna novae-zealandiae* (dark hover fly, Diptera: Syrphidae). I tested the hypotheses that: (1) key pollinators have different, species-specific behavioural patterns in relation to dominant environmental and structural variables in the crop, and (2) variation in pollinator behaviour throughout the growing season is dependent on changes in crop flowering phenology. To address these hypotheses, key pollinators were individually tracked and their behaviours recorded, and these were analysed in relation to variation in microclimate (light, relative humidity, temperature and wind speed), crop phenology (plant density and number of flowers per square meter) and the relative abundance of pollinators competing for flower resources.

MATERIALS AND METHODS

Study sites

The study was carried out in a single *Brassica rapa chinensis* (Pak-choi) crop of 50 × 50 m in size, located at Lincoln, Canterbury, New Zealand. The crop was drilled from seed on 12 September 2006 at 15 cm spacing and a depth of 2 cm, with a sowing rate of 2 kg seeds ha^{-1}. Trifluran herbicide was applied at 1.7 litres ha^{-1} to control weeds. The soil type was Wakanui Silt Loam. The amount of seed sown was 200 kg seeds ha^{-1}. Weeds were controlled with Trifluran at 1.7 litres ha^{-1}. Fertilisation was applied according to common practices used in Crop and Food Research before planting (the details of rate of application and fertilizer composition are commercially sensitive and are not available for release).

Crop development

The crop was monitored at weekly intervals over six weeks from the time the first leaves appeared on the emerging seedlings (November 2006), until seeds were formed on mature plants (January 2007). On each sampling date, plant structural data were recorded within a 5-m radius area at the centre, and at each of the four corners of the crop, in the vicinity of a flight interception trap (‘window trap’) at each location. Three 1-m^2 quadrats were sampled at each of the five window trap locations (giving a total of 15 samples per week), and plant and flower density measurements were recorded. The number of plants in each 1-m^2 sample was recorded, and the number of inflorescences per plant was estimated by counting the inflorescences on 10 randomly selected plants. The numbers of buds (with stigma and anther not yet visible), open flowers (with petals partially to fully open with stigma and anther exposed) and old (senescent flowers, with petals becoming necrotic) flowers per inflorescence

were estimated by counting the numbers of each flower type in 10 randomly selected inflorescences within each of the 10 plants. Plant height was measured with a measuring tape from the soil surface to the top of the tallest inflorescence, on five plants in each quadrat. These variables were recorded weekly until the flowering phase had finished.

Pollinator abundance

Pollinator abundance was measured with five window traps (see Figure 2.1 in Chapter 2) located at the centre and in each of the four corners of the crop. The window trap consisted of a grey 6 L plastic tray that supported a transparent Perspex window. The Perspex pane that ran lengthways along the tray had dimensions of 36.4 cm wide by 27.0 cm high (tapering at the tray base to 34.7 cm), while the pane running perpendicular to this was 23.8 cm wide by 27.0 cm high (tapering to 21.8 cm wide at the tray base). Four long stakes (1.2 m aluminium coated with green plastic) were hammered into the ground at a height that was just below the height of the crop flowers and in a pattern that matched the trap dimensions. The grey plastic tray was attached to the stakes using 15 cm long copper tubing to connect the stakes with the plastic tray joiners. The window trap was then placed on top to ensure that it was positioned at the same height as the flowers.

The tray was filled with 1 L of water containing detergent. The detergent was used to reduce the surface tension of the water to ensure efficient capture of insects. The traps were left for five days, and then the insects were collected and placed in labelled vials containing 70 % ethanol. In the laboratory the insects were sorted to species and the capture rates of the five key pollinators considered in this study were recorded each week. Total pollinator abundance was defined as the absolute frequency of all five species of pollinators pooled together.

Pollinator activity

On each of the six weekly sampling dates, nine randomly-selected free-living individuals of each of the five key pollinator species were followed for approximately three minutes each to determine foraging patterns under natural field conditions. For each individual insect, the numbers of flowers visited on each plant and the flight distances between successive flowers were recorded using an Olympus WS-100 digital voice recorder and a Sony Handycam DCR-HC85E digital video recorder. Flight distances were estimated visually and compared to measured distances from time to time. Each observation was performed about 1 m away from the insect, taking care to move slowly to avoid disturbing the insect's natural behaviour. A closer observation of the flowers was made at distances of approximately 10 cm to record

smaller insects. The nine insects of each of the five selected species were recorded as they were encountered, in no fixed sequence, at different times of the day, until measurements for all 45 insects had been completed on the same day when it was possible. Flower visitation data for each insect were converted into four distinct metrics measuring two components of average visitation rate and two components of variability in visitation rate, as depicted in Figure 3.1. The rate of movement through space was determined as total distance travelled by the insect during sampling divided by total time. The rate of flower visitation was determined as the total number of flowers visited during sampling divided by total time. Variability in movement was determined as the coefficient of variation (standard deviation divided by the mean) of the distances moved from flower to flower during the sampling period. Variability in flower visitation was determined as the coefficient of variation of times spent in each flower during the sampling period.

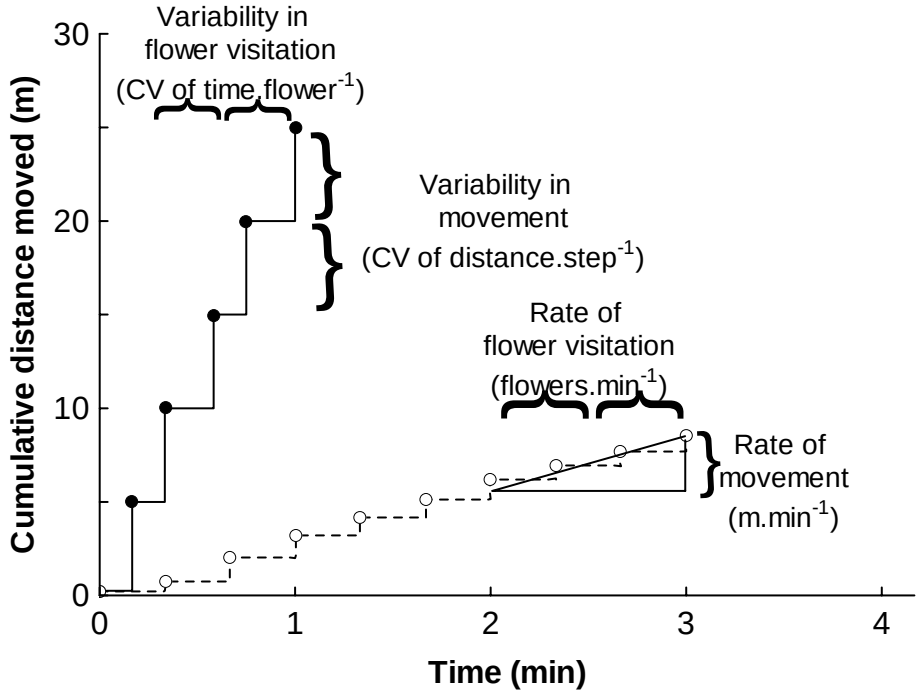


Figure 3.1. Conceptual diagram explaining how pollinator activity was measured. There are two variables describing average behaviour (rate of movement through space and rate of flower visitation through time) and two variables describing variability in behaviour (variability in distance moved between flowers and variability in flowers visited per unit time). CV indicates Coefficient of Variation.

Daily and seasonal microclimatic variation

Microclimatic variables were measured immediately after recording each insect. A Silva Windwatch was used to measure wind speed readings in km h^{-1}, a Thermo-Hydro recorder to

measure temperature (ºC) and relative humidity (%), and a Daystar meter (Daystar, inc.) to measure light intensity (W m^{-2}) with the meter oriented towards the sun.

Statistical analyses

General linear models (GLM) were used to determine whether crop and microclimatic variables significantly influenced the four measured pollinator behavioural variables. Initial GLM models were conducted in order to test whether the different species actually had different behavioural responses (i.e. all species in the same model). Separate GLM models were then used to test each of the four behavioural response variables, for each species. Following analyses, the residuals were tested for normality and homogeneity of variances, and data transformations were performed as necessary to meet the underlying statistical assumptions of the models used.

The first step in identifying the minimum adequate model describing significant variation in pollinator activity was to account for variation in pollinator activity as a result of varying conditions at the time of day that individual insects were observed. To do this, microclimate variables (wind speed, relative humidity, light and temperature) were incorporated one at the time in order of importance (proportion of variance explained) into the GLM, using type I sums of squares. Once the effect of variation in microclimate on behavioural variables was accounted for, the 'days after planting' variable was added into the model to test whether there was significant variation in pollinator activity throughout the season, over and above variation that could be explained by differences in microclimate at the time of day the insect was sampled. The 'days after planting' effect could potentially be due to a range of driving variables related to crop phenology. On each of the six sampling dates, a range of crop structure and flowering phenology variables provided potential explanatory power for the 'days after planting' effect. Because crop and pollinator abundance variables were only recorded once on each date, and there were only six dates sampled, there were only five available degrees of freedom to partition the potential drivers of the 'days after planting' effect. Therefore, variance partitioning was used to break down the proportion of the variance in 'days after planting' that were explained by just three variables, plant density, flower density and total pollinator abundance (Sokal and Rohlf 1995). Plant density (m^{-2}) was selected because it could have an important structural effect on movement of small insect, and it was not strongly correlated with any measure of flower density (all $P > 0.05$). The density of open flowers (m^{-2}) was selected as a measure of flower density in the crop because it is the most important variable indicating nectar availability, and it was highly significantly correlated with all other flower variables (all $P < 0.05$). Total pollinator abundance was

selected as a measure of pollinator activity in the area which could potentially interfere with individual behavioural responses, and it was strongly correlated with the abundances of all individual pollinator species (all $P < 0.05$). All statistical analyses were performed using Statistica v.7 software (StatSoft 2004).

RESULTS

Crop phenology

The crop started flowering at day 48 (November 2006), and flowering was completed by day 90. The number of plants per square meter increased steadily from approximately 28 plants m^{-2} at day 48 to approximately 36 plants m^{-2} by day 90. Plant height followed an asymptotic pattern with fast development up to day 62, and then growth progressively decreased, reaching a plateau (about 110 cm plant height) after day 76 (Figure 3.2.a). Flower density approximately followed a bell-shaped distribution, with a peak being observed at day 73 (Nov 24, 2006) (Figure 3.2.b).

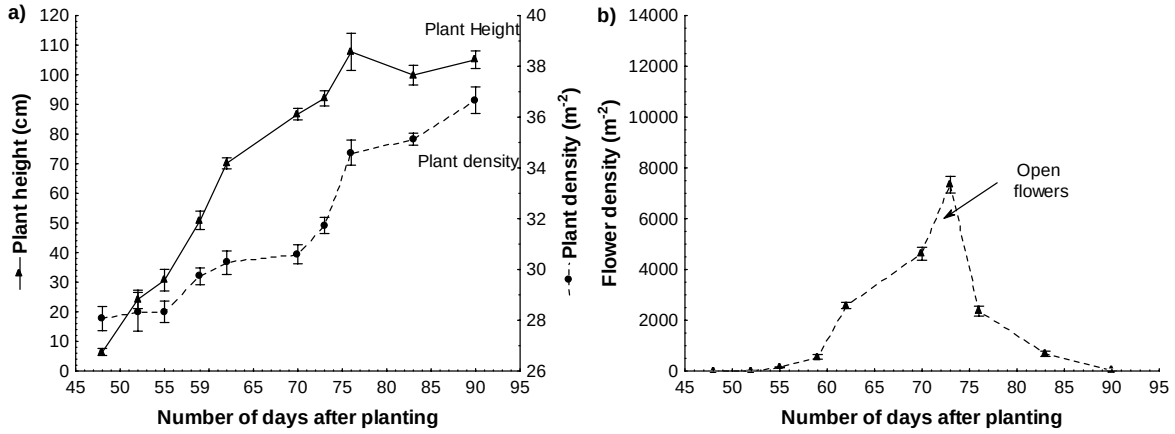


Figure 3.2. a) Crop density and plant height from the time of planting (September 12, 2006; datum zero) in a *Brassica rapa chinensis* crop at Lincoln, Canterbury. **b)** Flower density (flowers m^{-2}) at different stages of crop development. Values are presented as means (± 1 SE).

Variation in behavioural activity among pollinators

The rate of movement varied on average almost five-fold among key pollinators (Appendix 3.1). *Bombus terrestris* exhibited the greatest average rate of movement (473 ± 29 cm min^{-1}) and *L. sordidum* the least (97 ± 7 cm min^{-1}). *Apis mellifera* (336 ± 25 cm min^{-1}), *E. tenax* (240 ± 16 cm min^{-1}) and *M. novae-zelandiae* (240 ± 28 cm min^{-1}) exhibited average movement

rates between these two extremes. Overall, the rate of movement decreased with increasing crop development for all pollinators studied (Figure 3.3, Appendix 3.1).

The variability of movement between flowers was generally similar among key pollinators and stages of crop development. *Bombus terrestris* exhibited the greatest variability in movement (140 ± 10 %), and *M. novae-zelandiae* the least (93 ± 4 %). *Lassioglossum sordidum* (103 ± 4%), *A. mellifera* (119 ± 5 %), and *E. tenax* (129 ± 4 %) showed intermediate values between these two extremes.

As with the rate of movement, the rate of flower visitation varied on average five-fold among key pollinators. Overall, *B. terrestris* exhibited the greatest rate of flower visitation (25 ± 1.2 flowers min^{-1}) and *L. sordidum* (5.1 ± 0.3 flowers min^{-1}) the least. The rate of flower visitation by *A. mellifera* (14.8 ± 0.4 flower min^{-1}), *E. tenax* (13 ± 0.4 flower min^{-1}) and *M. novae-zelandiae* (8.9 ± 0.6 flower min^{-1}) lay between these two extremes. The rate of flower visitation varied on average by up to three-fold over the flowering season and key pollinators exhibited different patterns (Appendix 3.1). For *E. tenax* and *M. novae-zelandiae* the rate of flower visitation was greatest at the beginning of the flowering season and then progressively declined. For *B. terrestris*, *A. mellifera*, and *L. sordidum*, the rate of flower visitation increased progressively up to the peak of flowering and then declined (Appendix 3.1).

The variability of flower visitation rate varied on average less than two-fold among key pollinators and about two-fold across the flowering season. Overall, *L. sordidum* exhibited the greatest variability in flower visitation (75 ± 3 %), and *A. mellifera* the least (42 ± 3 %). Variability of flower visitation rate for *B. terrestris* (43 ± 1%), *E. tenax* (44 ± 2 %) and *M. novae-zelandiae* (64 ± 4 %) lied within this range.

The observed variation in patterns of pollinator activity suggests that different pollinator species have differing foraging responses to changes in crop phenology. General Linear Models testing this hypothesis for each of the four behavioural responses showed that the main effects of species ($F_{4, 269} > 15.02$, $P < 0.001$) and days after planting ($F_{5, 269} > 5.31$, $P < 0.001$) as well as their interaction ($F_{20, 269} > 2.96$, $P < 0.001$) were highly significant for all behavioural variables, indicating different behavioural patterns between species with increasing crop development (Appendix 3.2).

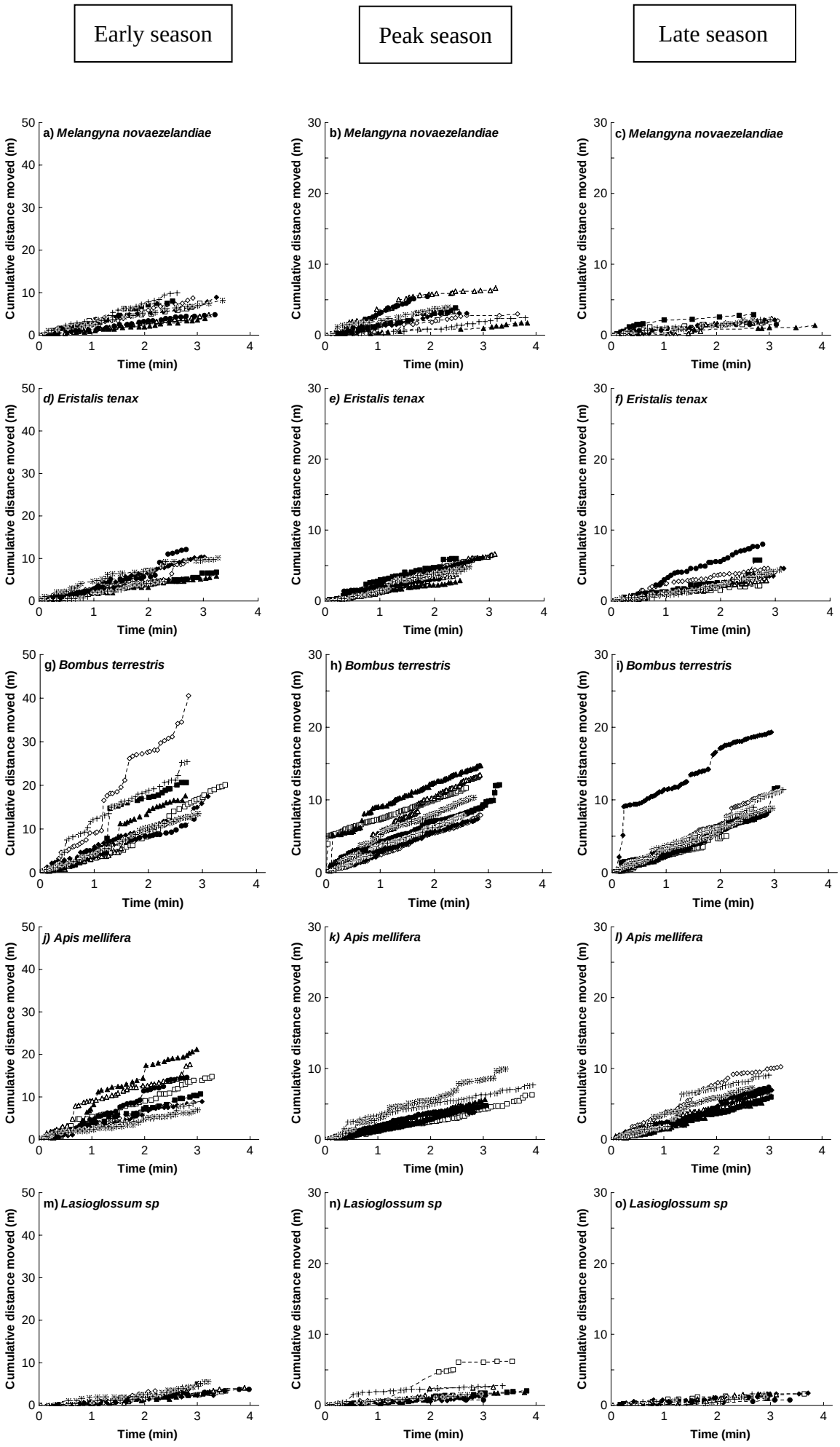

Early season
Peak season
Late season
a) Melangyna novaezelandiae
b) Melangyna novaezelandiae
c) Melangyna novaezelandiae
d) Eristalis tenax
e) Eristalis tenax
f) Eristalis tenax
g) Bombus terrestris
h) Bombus terrestris
i) Bombus terrestris
j) Apis mellifera
k) Apis mellifera
l) Apis mellifera
m) Lasioglossum sp
n) Lasioglossum sp
o) Lasioglossum sp
Cumulative distance moved (m)
Time (min)

Figure 3.3 (previous page). Movement trajectories for individuals of five key pollinator species during early, peak and late flowering of a *Brassica rapa chinensis* crop. Symbols represent the points at which an insect stopped to feed at a flower. Each line represents the movement trajectory of one of nine insects moving between flowers.

Determinants of the rate of movement

For four of the five species (*M. novae-zelandiae*, *A. mellifera*, *E. tenax* and *L. sordidum*), the rate of movement varied significantly throughout the season, after accounting for variation in microclimatic conditions at the time of sampling (Appendix 3.3). For example, the GLM model for *M. novae-zelandiae* (Table 3.1) showed that after accounting for significant variation in the rate of movement due to varying wind speed, temperature and light intensity at the time of sampling (explaining a combined total of 40.0% of variation in the data), there was still a highly significant effect of days after planting on the rate of movement ($F_{5, 45}$ = 13.881, r^2 = 0.364, $P < 0.001$). Variance partitioning showed that the majority of the days after planting effect for *M. novae-zelandiae* was explained by plant density (54.7 % of the days after planting effect; Figure 3.4a), flower density (32.6 %; Figure 3.4b) and pollinator abundance (0.4 %). Qualitatively similar results were obtained for *E. tenax*, *A. mellifera* and *L. sordidum*, with 27 – 35 % of variation in the data explained by microclimatic variation at the time of sampling, and 22 – 36 % of variation in the data explained by crop phenology (Appendix 3.3).

For *B. terrestris*, in contrast to the four specie described above, the rate of movement did not change throughout the season ($F_{5, 46}$ = 2.19, P = 0.072), after accounting for variation in microclimatic conditions at the time of sampling (Appendix 3.3).

Table 3.1. A General Linear Model testing variation in the logarithm of rate of movement of *Melangyna novae-zelandiae* between *Brassica rapa chinensis* flowers.

Source	d.f.	S.S.	M.S.	*F*	*P*
Wind speed	1	2.750	2.750	14.829	<0.001
Temperature	1	1.130	1.130	6.092	0.017
Light	1	10.289	10.289	55.484	<0.001
Days after planting	5	12.870	2.574	13.881	<0.001
Plant density	1	7.038	7.038	8.886	
Flower density	1	4.199	4.199	5.302	
Pollinator abundance	1	0.049	0.049	0.062	
Error within Days after planting	2	1.584	0.792		
Error	45	8.345	0.185		
Total	53	35.384			

Although microclimate had a strong influence on the rate of movement for all key pollinators, the importance of different microclimatic variables varied between species (Appendix 3.3). Light ($P < 0.032$), temperature ($P < 0.01$) and wind speed ($P < 0.001$) explained significant variation in the rate of movement of *M. novae-zealandiae*, *E. tenax* and *A. mellifera*, whereas for *L. sordidum* relative humidity ($P < 0.001$) and light ($P < 0.001$) were the most important variables. Wind speed ($P < 0.024$) and relative humidity ($P < 0.006$) explained significant variation in the rate of movement of *B. terrestris*.

Days after planting, as a surrogate for crop phenological development, had a significant effect ($P < 0.003$) on the rate of movement of all species, except *B. terrestris* ($P = 0.072$). The days after planting effect were differentially explained by plant density, flower density and total pollinator abundance according to species. For *M. novae-zelandiae* and *E. tenax*, plant density explained the major part of the days after planting variance (49 – 55 %) (Figure 3.4a), followed by flower density (2.5 – 33 %) (Figure 3.4b) and pollinator abundance (0.4 – 1.6 %). However, variation in the rate of movement of *A. mellifera* with crop development was determined more by flower density (77.4 %) than plant density (18.7 %), and variation in the rate of movement of *L. sordidum* with crop development was determined more by the total abundance of other active pollinators (Appendix 3.3), with no strong effect of flower density (Figure 3.4; Appendix 3.3).

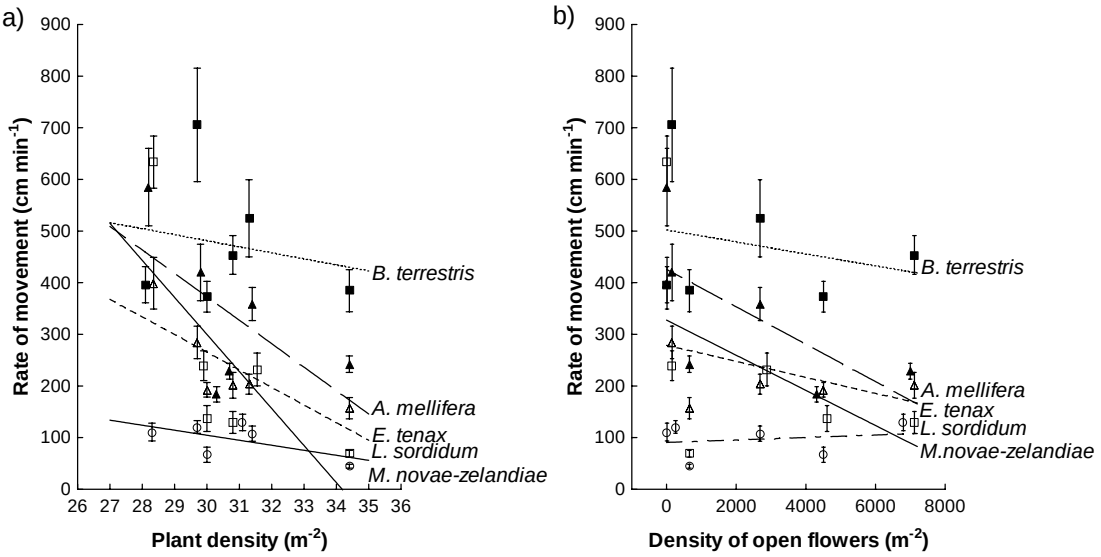


Figure 3.4. Rate of movement of key pollinators during crop development, expressed in terms of **a)** plant density and **b)** density of open flowers.

Determinants of rate of flower visitation

In separate GLM models for each species, microclimate had a strong influence on the rate of flower visitation for all key pollinators, but the relative importance of different microclimate variables differed between species. Light ($P < 0.016$) and temperature ($P < 0.034$) explained significant variation in the rate of flower visitation for *M. novae-zelandiae* and *E. tenax*, while for *B. terrestris*, *A. mellifera* and *L. sordidum*, light ($P < 0.001$), relative humidity ($P < 0.001$), temperature ($P < 0.002$) and wind speed ($P < 0.001$) explained significant variation in rate of flower visitation.

Days after planting, as a surrogate for crop development, had a significant effect ($P < 0.001$) on the rate of flower visitation of all species except for *B. terrestris* ($P = 0.06$). Most of the days after planting effect were differentially explained by plant density, flower density, and pollinator abundance. For *M. novae-zelandiae*, *E. tenax* and *L. sordidum* plant density explained the greatest proportion of the days after planting effect (18 – 76 %) (Figure 3.5a) followed by flower density (0.1 – 25 %) and pollinator abundance (2.5 – 17 %). *A .mellifera* was atypical in that flower density explained the greatest proportion of the days after planting variance (69 %) (Figure 3.5b), followed by plant density (26 %) and pollinator abundance (2 %).

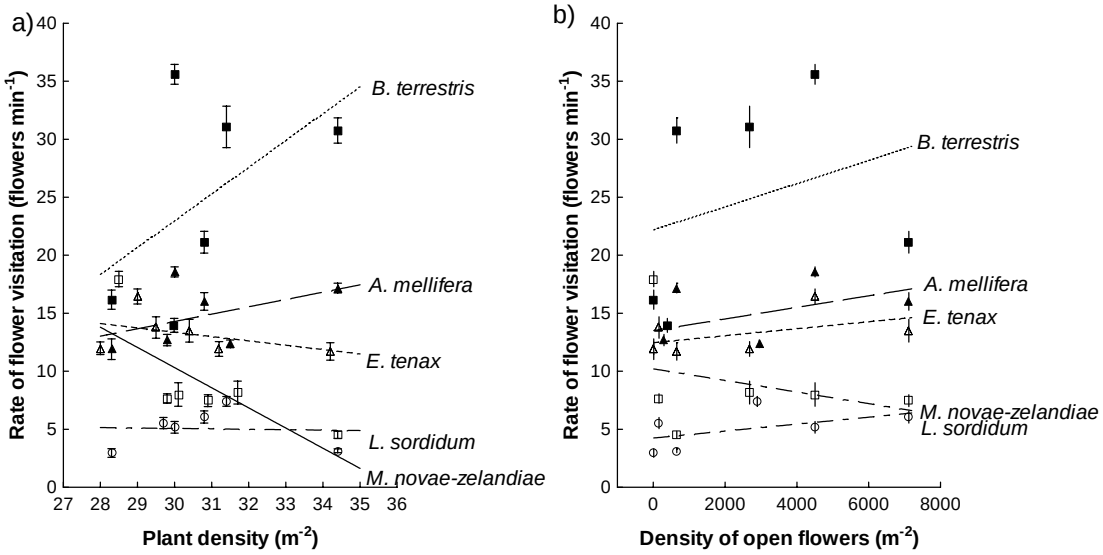


Figure 3.5. Rate of flower visitation of key pollinators during crop development, expressed in terms of **a)** plant density and **b)** density of open flowers.

Determinants of variability in movement

In separate GLM model for each species, microclimatic variables had a significant effect on the variability of movement between flowers for all key pollinators, except for *L. sordidum* (Appendix 3.4). However the importance of different microclimatic variables varied between species. Light ($P < 0.027$) explained significant variation in the variability of movement between flowers for *M. novae-zelandiae, B. terrestris* and *A. mellifera*. For *E. tenax,* relative humidity ($P < 0.021$) explained the rate of flower visitation, whereas for *M. novae-zelandiae* relative humidity ($P < 0.001$), additional to light, significantly influenced the rate of flower visitation.

Days after planting, as a surrogate for crop development, had a significant effect ($P < 0.001$) on the variability of movement only for *M. novae-zelandiae* (Appendix 3.4, Figure 3.6), and in this case the days after planting effect was mainly explained by plant density (15%), flower density (10%), and pollinator abundance (3%).

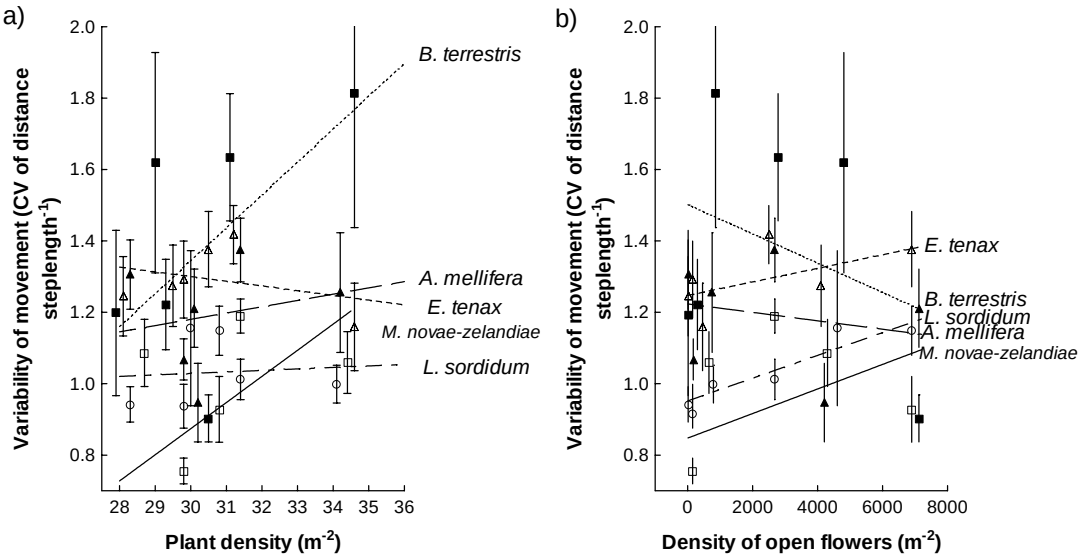


Figure 3.6. Variability in the rate of movement of key pollinators during the crop development, expressed in terms of **a)** plant density and **b)** density of open flowers.

Determinants of variability in flower visitation

In separate GLM models for each species, microclimate had a strong influence on the variability in flower visitation for all key pollinator except *E. tenax*. Wind speed ($P < 0.019$) explained significant variation in flower visitation for *M. novae-zelandiae* and *A. mellifera*. Only light ($P < 0.003$) explained variability in flower visitation for *B. terrestris*, and only relative humidity ($P < 0.03$) influenced the variability in flower visitation of *L. sordidum.*

Days after planting, as a surrogate for crop development, had a significant effect on the variability of flower visitation for all species ($P < 0.03$) except *B. terrestris* ($P = 0.17$). For *M. novae-zelandiae*, the days after planting effect was mainly explained by flower density (41 %) (Figure 3.7 b) and then by plant density (19 %) and pollinator abundance (1%). For *E. tenax*, plant density (20 %), flower density (3 %) and pollinator abundance (1 %) explained the days after planting effect. For *L. sordidum*, plant density (45 %) (Figure 3.7a), flower density (5 %) and pollinator abundance (0.2 %) explained the days after planting effect. In the case of *A. mellifera*, the day after planting effect was explained by plant density (56 %), flower density (24 %) and pollinator abundance (2 %).

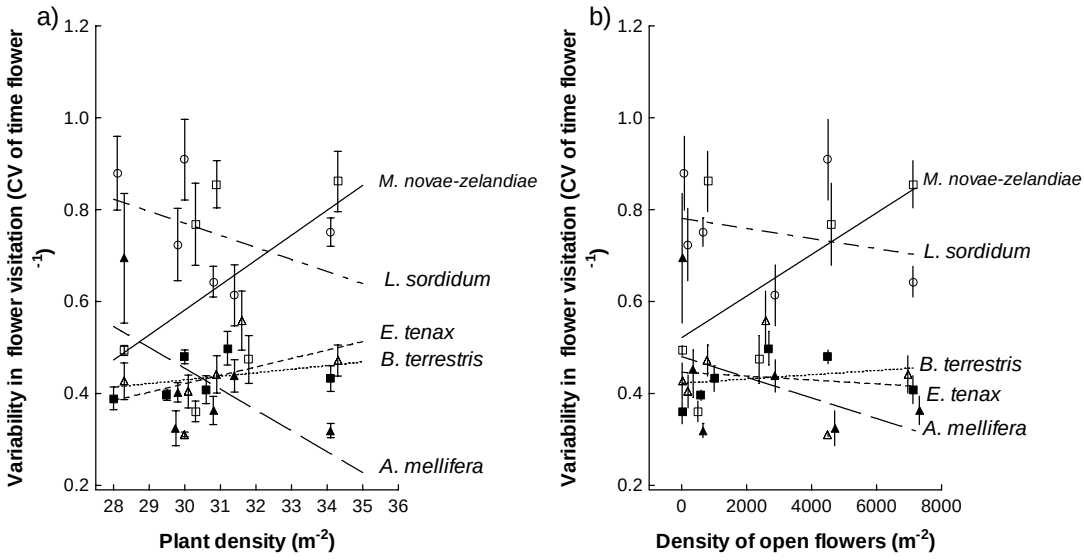


Figure 3.7. Variability of flower visitation by key pollinators during crop development, expressed in terms of **a)** plant density and **b)** density of open flowers.

DISCUSSION

There is relatively little information available on temporal variation in behavioural responses of different pollinator species to changing floral phenology throughout the season (Kwak 1997). After accounting for the differential effects of variation in daily microclimate variables, such as light, relative humidity, temperature and wind speed, on the foraging activity of the five key pollinator species, this study showed that there were strong species-specific differences in the foraging behaviour of *Brassica* pollinators with changing crop phenology. Temporal variation in the rate and variability of movement between flowers, and the duration and variability in time spent on each flower, throughout the growing season, differed markedly between pollinator species.

Microclimatic determinants of variation in pollinator foraging activity

The foraging behaviour of pollinators is strongly influenced by abiotic factors (Herrera 1995). Light, temperature and wind speed explained significant variation in the rate of movement between flowers for *M. novae-zealandiae*, *E. tenax* and *A. mellifera* in this study, and similarly Vicens & Bosch (2000) reported that *A. mellifera* is very active at temperatures higher than 14°C and solar radiation over 300 Wm^{-2}. For *B. terrestris*, wind speed and relative humidity explained significant variation in the rate of movement, with similar results reported previously by Peat & Goulson (2005).

Microclimate influenced the rate of flower visitation per minute for all key pollinators in my study. For *B. terrestris*, *A. mellifera* and *L. sordidum*, light, relative humidity, temperature and wind speed explained significant variation in rate of flower visitation. Totland (1994) found that the rate of flower visitation is restricted in Diptera when the wind speed is high, and he demonstrated that there is a negative correlation between visitation activity and wind speed. Comba (1999) reported that Apidae generally limit flower visitation to avoid being carried away and disorientated on windy days. My results are congruent with McCall & Primack (1992) who reported that high visitation rate was related to warm temperatures, high levels of light, low wind speed and low relative humidity.

I factored out week to week variation in microclimate before testing the effects of changing crop phenology on pollinator activity. This could potentially remove some of the explanatory power to test the effects of changing crop phenology on pollinator activity, because there is likely to be intercorrelation between increasing temperatures through the season and increasing flower density. However in order to separate the effects of microclimate and crop phenology on pollinator behaviour would require much greater sampling intensity at different times of the day and on multiple days per week, which was not logistically feasible in this study.

The relative influence of crop phenology on pollinator foraging activity

The analysis of behavioural variables using general linear models allowed me to divide key pollinators into two groups: *B. terrestris*, which showed no change in the rate of movement with changing crop phenology (after accounting for climatic variation) versus all other species (*A. mellifera*, *E. tenax, M. novae-zelandiae* and *L. sordidum*), which did show a significant change in foraging activity throughout the season. *Bombus terrestris* also stands out as having the greatest rates of flower visitation and the greatest rates and variability of movement among all key pollinators studied. For all these reasons, *B. terrestris* is one of the key pollinators studied in this research which can contribute most to gene flow. Other authors

cited *Bombus* as a risky pollinator for gene escape (Waddington 1981, Rasmussen & Brodsgaard 1992, Walklate *et al*. 2004).

For all those species for whom their behaviour was influenced by crop phenology (*A. mellifera*, *E. tenax*, *M. novae-zelandiae* and *L. sordidum*), variance partitioning revealed that plant density, flower density and the abundance of other insects contributed to explaining pollinator behaviour, although there was little statistical power to test the significance of these effects due to the limited number of sampling times throughout the season. Nevertheless, the proportion of total variance explained by crop phenology was generally lower than the proportion explained by microclimate.

Species-specific patterns of pollinator foraging activity

I found a highly significant Species × Days after planting interaction effect for all four behavioural variables (rate of movement, variability of movement, rate of flower visitation and variability in flower visitation), implying dissimilar behavioural patterns between species with increasing crop development. For instance, the rate of flower visitation in *E. tenax* was greatest during the early flowering period and then decreased during the season, while generally for Hymenoptera (particularly *A. mellifera* and *L. sordidum*) the rate of flower visitation increased until the peak of flowering and stayed relatively constant thereafter (Thomson 1981, Real & Rathcke 1991, Totland 1994). The latter case has been observed by several authors (e.g. Totland 1994, Kwak 1997) and attributed to pollinators getting used to visit only a limited area at the peak of flowering and then remaining faithful to the same area even after flower density declines.

The study showed that as the plant density and open flower density increased, the average flower visitation rate increased for most species, while the average distance moved between flowers decreased significantly. Several studies have shown the relationship between flower density and pollinator activity (Schmitt 1983, Feinsinger *et al*. 1991, Kunin 1993). General linear models in this study showed that after accounting for microclimate, the rate of flower visitation increased with flower density except for *B terrestris*. Thomson (1981), Real & Rathcke (1991) and Totland (1994) described a positive correlation between flower density and the rate of flower visitation, which is congruent with my results for four out of five key pollinators.

Another key finding of this study is that most species would respond more to plant density than to flower density. Plant density had an important structural effect on movement and flower visitation rates for all insects except *B. terrestris*. Higher plant densities seem to be more attractive to pollinators than sparse but dense patches of flowers (Sih & Baltus 1987,

Ågren 1996, Chittka & Thomsom 2001, Feldman 2006). The contribution of pollinator abundance to explain behaviour was generally far lower than the contribution of plant and flower density, except for *L. sordidum* where pollinator abundance contributed similarly to plant density in explaining its behaviour. This might be due to *L. sordidum* being the smallest of all key pollinators, and all other pollinators exercising their territoriality.

Implications for gene flow within and between crops

The rates of movement of *B. terrestris*, and to a lesser degree *A. mellifera*, were far greater than those of other key pollinators, implying that they can potentially contribute to gene escape to others crops. However, pollen transport is not necessarily indicative of outcrossing, because it depends heavily on pollen viability (see Chapter 4). Variability of movement may be considered as an indicator of unexpectedly long-distance movements, which potentially can involve pollen exports outside the crop. *B. terrestris* had the greatest variability in the rate of movement, so there is a large chance that this species contributes to gene escape from crops.

According to Newstrom *et al*. (2003), most pollen flow happens over short distances. *Melangyna novae-zelandiae*, *E. tenax* and particularly *L. sordidum* moved only short distances (as opposite to *B. terrestris* and *A. mellifera*) and therefore might be major contributors to gene flow within short distances. This short movement may imply gene flow inside the crop and the risk of cross pollination with weeds (Godt & Hamrick 1993). Overall, differing patterns of long and short distance movements vary throughout the season, following crop phenology and being maximum at the peak of flowering.

In summary, I assessed the behaviour of key pollinators in *Brassica rapa* crops in order to test whether such behaviour was species-specific and influenced by crop phenology, and whether some pollinators were more risky for gene flow than others. The study showed that pollinator behaviour is highly variable between species, with all key pollinators except *B. terrestris* being influenced by crop phenology after accounting for microclimatic variation, and that *B. terrestris* and *A. mellifera* are long distance pollen carriers while at the other extreme *L. sordidum* may contribute to gene flow only over small distances.

Chapter Four

Arthropod pollen loads and viability of pollen in a *Brassica rapa chinensis* crop

INTRODUCTION

In order to understand patterns of outcrossing and genetic change in plant populations, breeders, geneticists and evolutionary biologists have studied the patterns and processes of pollen movement in the landscape (Bateman 1947, Ellstrand 2003). For some of the most important crop species, these patterns of pollen movement are mediated by insect pollinators. There is increasing interest in the role that insect-mediated pollen transfer plays in the spread of transgenes from commercial cultivars into landraces or wild relatives (and vice versa), with the associated risks of genetic erosion and increased weediness (Rodgers & Parkes 1995). Despite this, there is still remarkably little known about pollen transfer by different species of insects in most crops. Certainly, pollen movement has been measured over several hundreds of metres within fields (Scheffler *et al*. 1995, Hall *et al*. 2000, Beckie *et al*. 2003), and a number of kilometres between fields (Rieger *et al*. 2002, Ramsay *et al.* 2003, Devaux *et al*. 2007). However, the proportion of these pollen grains that remain viable during transport is almost never tested (Richards *et al*. 2005). Pollen viability has been extensively studied in some plant species (Govila & Rao 1969, Luyt & Johnson 2001, Ranito-Lehtimäki 1995, Ramsay *et al*. 1999), but the results of these studies have not been suited for predicting the importance of pollen viability in transgene spread (Bots & Mariani 2005). Only in a small number of cases has pollen viability been investigated in the field after dehiscence to the environment and subsequent transport out of the crop (Conner & Zangori 1997, Aylor 2003).

Pollen management is an emerging field concerned with limiting transgene flow across different crops (Garcia *et al*. 1998). Pollen dispersal depends on the behaviour of flower visitors, which is explained by a complex array of factors such as cultivar type, plant arrangement, local topography, environmental conditions, plant density, and availability of pollen and nectar (Eisikowitch 1981, Rieger *et al*. 1999, Pierre 2001, Légère 2005, Ceddia *et al*. 2007). Other factors to be considered in pollen dispersal are synchrony of flowering, density of donor and recipient populations, and the quality and viability of pollen dispersed, among others (Ingram 2000, Eastham & Sweet 2002, Légère 2005, Ceddia *et al*. 2007).

Gene flow is of particular concern in *Brassica* crops, because most species have a substantial outcrossing rate (Hüsken & Dietz-Pfeilstetter 2007). Although *Brassica* pollen is large, 32-35 µm (Delaplane & Mayer 2000, Hüsken & Dietz-Pfeilstetter 2007), and too heavy to be transported by air currents (Richards *et al*. 2005), it is sticky, which allows all flower visitors to become potential pollinators. *Brassica* also produces large amounts of pollen (9.3 ± 0.5 kg pollen ha^{-1}) (Westcott & Nelson 2001) which makes it particularly suitable to study gene flow by pollen transportation (Légère 2005, Ceddia *et al*. 2007). However, *Brassica* pollen only remains fully viable for up to 72 hours (Govila & Rao 1969), after which viability decreases drastically over 4 to 5 days (Ranito-Lehtimäki 1995). Consequently, there are two very important components to insect-mediated gene flow that need to be distinguished, and these are the total quantity of pollen transported and the viability of that pollen, over varying distances.

In terms of the total quantity of pollen transported, it is widely recognised that insect pollinators typically transfer only about 1% of the total quantity of pollen produced by a single flower (Galen & Stanon 1989, Thomson & Thomson 1989, Young & Stanton 1990, Conner *et al*. 1995), and that only a small fraction of this pollen is eventually dislodged in successive visits to other flowers (Robertson 1992). The efficiency of pollen carry-over between flowers can vary widely among pollinator species (Primack & Silander 1975, Schemske & Horvitz 1984, Herrera 1987, Conner *et al*. 1995, Mayfield *et al*. 2001, Ivey *et al*. 2003, Hayter & Creswell 2006). Flower visitors can vary in their pollination efficiency due to differences in body size (Adler & Irwin 2005) and in their ability of pick up and deposit pollen (Conner *et al*. 1995), while plant species can vary in the capacity of their flowers to donor pollen to flower visitors (Ganders 1979). For example, studies have shown that bees transport more pollen than butterflies (Fishbein & Venable 1996). Bees transport more pollen grains (10,000 – 25,000) on their bodies than perhaps any other insect (Degrandi- Hoffman *et al*. 1992), with pollen grains sticking to the body hairs and the majority staying relatively well protected from environmental conditions (Bots & Mariani 2005). In other cases, native pollinators have been shown to transfer even more pollen than honey bees in some crops, such as almond, onion and cranberry (Bosch & Blas 1994, Cane & Schiffhauer 2003). Kumar *et al*. (1985) reported that *Eristalis tenax* carried significantly higher numbers of pollen grains than *Apis* spp. and other insect visitors on onion. Importantly, though, Sahli & Conner (2007) reported that in some instances the least efficient pollinator species in terms of pollen transportation in *Brassica* crops may be the most important pollinator species in terms of plant reproduction, simply because it is the most frequent flower visitor.

Having a large quantity of pollen transported between flowers does not necessarily ensure pollination, because the pollen of most plant species only remains viable for a short period of time. Few studies have measured how much viable pollen is transported by pollinating insects, such as bees (Bots & Mariani 2005), but these studies have typically found that pollen viability decreases during transportation by insects (Ramsay *et al*. 1999, Légère 2005) because viability is strongly influenced by environmental conditions. For example, Vaissiere *et al*. (1996) found that the viability of *Cucumis melo* pollen carried by *Apis mellifera* was substantially lower (28%) than the viability of pollen of the same maturity on the anthers of flowers prior to insect transport (80%). Low atmospheric relative humidity, high temperatures, UV-B radiation and long day length have all been found to negatively affect pollen viability (Ockendon & Gates 1976, Heslop-Harrison 1979, Shoper *et al*. 1987). The rate of decrease in pollen viability in response to adverse environmental conditions is related to the condition of hydration of the pollen at dehiscence (Nepi *et al*. 2001). Pollen develops in a locus nourished by locular fluid and this liquid hydrates the anther and the pollen before dehiscence (Pacini *et al*. 1997). Franchi *et al.* (2002) found that pollen normally has over 30% water content at dehiscence in 40 angiosperm families tested, and Nepi *et al.* (2001) showed that when pollen is only partially hydrated at dehiscence, viability can drop to as low as 10%. Pollen resistance to dehydration (Nepi & Pacini 1993, Lisci *et al*. 1994) involves a range of mechanisms, including varying concentrations of cytoplasmic disaccharides and oligosaccharides that act as membrane stabilizers (Hoekstra *et al*. 1988, Buitink *et al*. 1996, Speranza *et al*. 1997), and aspects of the morphology of the pollen grain, such as the number of pores in the membrane (Dajoz *et al*. 1991). As a result, the ability for pollen to survive environmental exposure varies between plant species (Nepi *et al.* 2001, Pacini *et al*. 1997), with pollen of insect-pollinated plant species typically having longer viability than in other plant species because the pollen must wait for a pollinator to pick up the pollen and then transport it to another flower (Pacini *et al*. 1997).

In addition to the condition of pollen at dehiscence, there are also pollinator-specific differences in the rate of reduction in pollen viability during transport. For example, Wagner (2000) concluded that one reason why ants may not be effective pollinators, even though they can transfer pollen from plant to plant, is because many species of ants secrete antibiotic substances that reduce pollen viability. Similarly, among *Brassica* flower visitors, Harris & Beattie (1991) found that some species of bees and wasps had higher levels of antibiotic secretions than other species, and this was a potential reason for differences in the viability of pollen carried by different species. In the moth *Helicoverpa armigera*, Richard *et al*. (2005) reported that the viability of pollen carried on the proboscis declined due to the secretion of a

toxin, suggesting that the large quantities of pollen transported by this species (Del Socorro & Gregg 2001) may not be an indicator of pollination effectiveness.

The aim of this study was to test whether total pollen loads and relative pollen viability transported by five key pollinator species inside and outside *Brassica rapa chinensis* crops varied with flower phenology.

MATERIALS AND METHODS

Brassica flower structure

Brassica rapa belongs to the Cruciferae family with inflorescences being elongated racemes with shiny yellow flowers. Flowers stand well above the unopened younger buds. *Brassica* flowers have an open flower structure with stigma and stamen exposed (Downey *et al.* 1980). Most *Brassica* are self-incompatible and the stigma becomes receptive two days after the flower opens and the pollen remains viable for seven days, although the petals and sepals shed on the fourth day (Free 1970).

Study sites

The study was conducted in two *Brassica rapa chinensis* (Pak-choi) crops, situated at Lincoln, in Canterbury, New Zealand. The dimension of each field was 50 × 50 m. The crops were seed drilled on 12 September 2006 (early season planting) and 10 November 2006 (late season planting), respectively. The amount of seed sown was 200 kg seeds ha^{-1}. Weeds were controlled with Trifluran at 1.7 litres ha^{-1}. Fertilisation was applied according to common practices used in Crop and Food Research before planting (the details of rate of application and fertilizer composition are commercially sensitive and are not available for release).

Measuring pollen viability in *Brassica* flowers

Both crops were monitored at weekly intervals from the time that flowers appeared in the fields. On each sampling date, eight newly-opened flowers were taken at random from inflorescences within a single randomly selected plant at each of five locations within the field (40 flowers in total). Flowers were placed in separate Petri dishes, and pollen viability was assessed on the same day of collection using the procedure described below. Pollen from the stamen of the flowers was removed with a small camel-hair paintbrush and then the paintbrush was cleaned and examined under the microscope to ensure no pollen was left on the bristles between samples. The pollen was placed on a microscopic slide to test the viability of *Brassica* pollen using the fluorochromatic reaction (FCR) procedure (Heslop-

Harrison *et al*. 1984). Fluorescein diacetate (FDA) (Sigma-Aldrich Co., cat. F-7378, Clayton, Vic), 0.02g, was mixed with 10ml of acetone. A 20% sucrose solution was made up and 5 ml removed into a separate container. The FDA solution was added drop by drop to this 5 ml of sucrose until persistent turbidity occurred. This solution was used within 30 minutes of mixing. One drop of the mixture of Fluorescein diacetate (FDA), acetone and sucrose was placed on the microscope slide containing the pollen, and the slide was immediately placed into a humid chamber for 10 min. This humid chamber was created by placing moist filter paper in a Petri dish. After 10 min a cover slip was placed over the sample, which was viewed under a fluorescence microscope (Excitation range Blue; Excitation Filter BP 450-490; Dichromatic mirror 510; Suppression filter LP515; magnification × 200), within 10 min of adding the cover slip. The optimal concentration of sucrose to use in the FDA technique varies between species (Heslop-Harrison *et al*. 1984), and for this reason concentrations of 15 % and 20 % were tested in preliminary experiments. No significant difference was found so 20 % sucrose was used. All the grains visible on the slide were scored as viable or not viable, and the percentage viability calculated. As can be seen in Figure 1, viable pollen fluoresced a bright green colour and the non-viable pollen remained dull in colour. For all flowers in which pollen viability was determined, pistil length was measured from the base to the tip of the stigma using a digital calliper. Pistil length is known to increase with flower age (Shykoff *et al*. 1997).

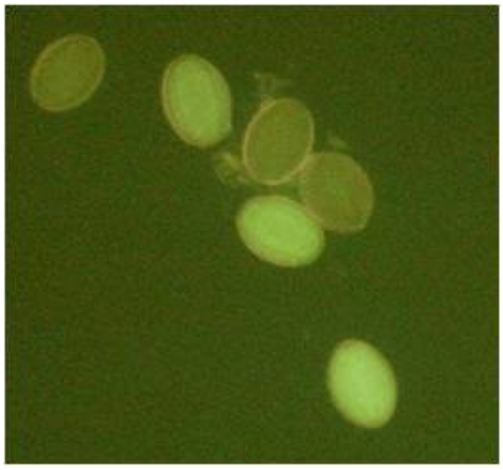


Figure 4.1 Viable (light) and non-viable (dark) pollen stained with a mixture of Fluorescein diacetate (FDA), acetone and sucrose under a fluorescence microscope (Excitation range Blue; Excitation BP 450-490; Dichromatic mirror 510; Suppression filter LP515; magnification ✕ 200).

Measuring the viability of pollen transported by key pollinators

Standardised pollen loads and pollen viability were determined for five key pollinator species, *Bombus terrestris* (bumblebee, Hymenoptera: Apidae), *Apis mellifera* (honeybee, Hymenoptera: Apidae), *Lasioglossum sordidum* (Hymenoptera: Halictidae), *Eristalis tenax* (drone fly, Diptera: Syrphidae) and *Melangyna novae-zealandiae* (dark hover fly, Diptera: Syrphidae). Nine insects per species were collected weekly after the first flowers appeared in the early and the late season crops. Insects were collected using sticky traps (Figure 4.2) placed inside the crop and 50 m outside the border of the crop. When sticky traps did not provide enough insects, additional insects were manually collected using nets. Sticky traps consisted of a yellow panel fixed vertically to metal poles, and then yellow paper was fixed to the panel with Velcro™ and covered with tanglefoot™ adhesive. Insects were collected from 9 am to 5 pm. Insects were rapidly killed by placing them into plastic vials containing tissue-paper soaked with ethyl acetate. In the laboratory, pollen was removed by rubbing the whole body with one 10 µl drop of a mixture of Fluorescein diacetate (FDA), acetone and sucrose (using a small camel-hair paintbrush), and depositing the pollen-FDA mixture on a clean microscope slide. It was not logistically possible to remove and count all of pollen that might have been on each of the insects captured. Instead, the count was carried out on five random 1-mm diameter circles on each microscope slide. All the grains of *Brassica rapa chinensis* and non-target pollen within these areas on the slide were counted, and scored as viable or not viable, and the percentage viability of *Brassica* pollen was calculated for each insect. To compare standardized pollen counts inside and outside the crop a t-test was used. I refer to this as a standardised measure of pollen load. The method for measuring pollen viability was the same as described above. For the first two weeks of insect collection (in the early-season planting), the pollen viability test was conducted immediately after the capture of all insects in the field. Subsequently, it was not logistically feasible to conduct the viability tests immediately, and therefore insects were stored at -80 °C until the viability test could be conducted (from 20 – 40 days later).

Figure 4.2 Sticky traps were placed inside and outside the field in order to catch insects and measure standardised pollen load and relative pollen viability.

Controlled experiment to test the effects of freezing preservation on pollen viability

Because not all pollen viability tests could be conducted on the day of sampling for the insect-transported pollen, there was the potential for confounding effects of -80 °C freezing on pollen viability. Therefore, long-term freezing preservation effects on pollen viability were tested under controlled conditions using pollen collected from plants grown in the greenhouse.

Brassica rapa chinensis seeds were planted in a tray of sand on 04 August 2006, and two plants were transplanted into each of 90 (2.5 litre) pots containing standard potting mix when they were at the four-leaf stage. Atmospheric temperature, light and humidity were affected by the greenhouse, but not instrumentally controlled. The first flowers appeared 45 days after re-potting, and at the peak of flowering all the flowers were harvested and placed in Petri dishes labelled with collection date and stored at -80 °C in a freezer. The first 40 flowers collected were tested for pollen viability on the day of harvesting. Thereafter, pollen viability in 40 randomly-selected frozen flowers was assessed each week for the first four weeks, and then each month for three months (a total of 120 days). The method used to measure pollen viability was the same as that described above.

RESULTS

Measuring pollen viability in *Brassica* flowers

Total and viable pollen counts per flower and pollen viability prior to dehiscence (range 40 – 70%) tended to decrease with crop development for both the early season and the late season crops (Figs 4.3 and 4.4). The late season crop started flowering much sooner after planting than the early season crop, and the standardised pollen count and pollen viability at the onset of flowering were generally higher in the late season crop.

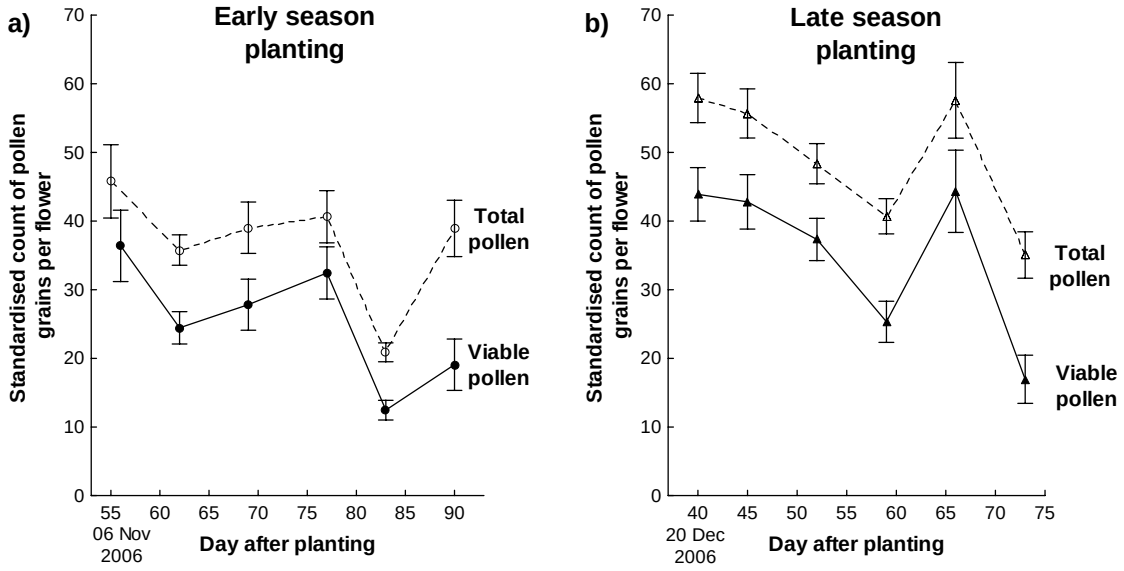


Figure 4.3 Standardised counts of total pollen and viable pollen in *Brassica rapa chinensis* flowers with changing crop phenology in early-season and late-season plantings. Values are presented as means (± 1SE) of 40 flowers at each date.

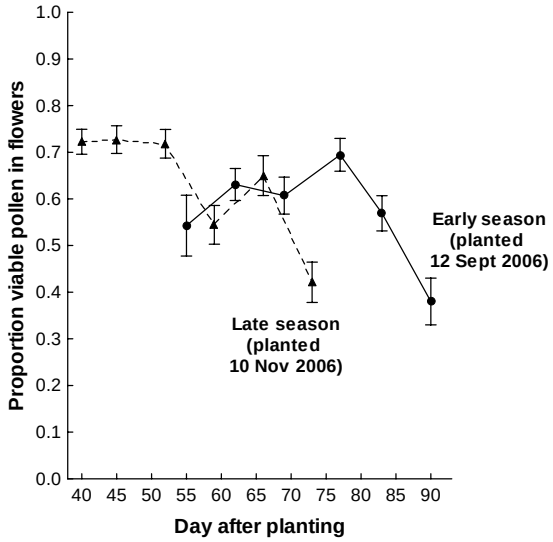


Figure 4.4 Proportion of viable pollen in *Brassica rapa chinensis* flowers with changing crop phenology in early-season and late-season plantings. Values are presented as means (± 1SE) of 40 flowers at each date.

At least part of the variability in pollen viability between flowers, and with changing crop phenology may have been due to flower age. Pollen viability varied dramatically between individual flowers, and there was a significant negative correlation between pollen viability and pistil length for the early season (r = - 0.64, n = 240, p <0.01) and the late season crop (r = - 0.29, n = 240, p <0.01).

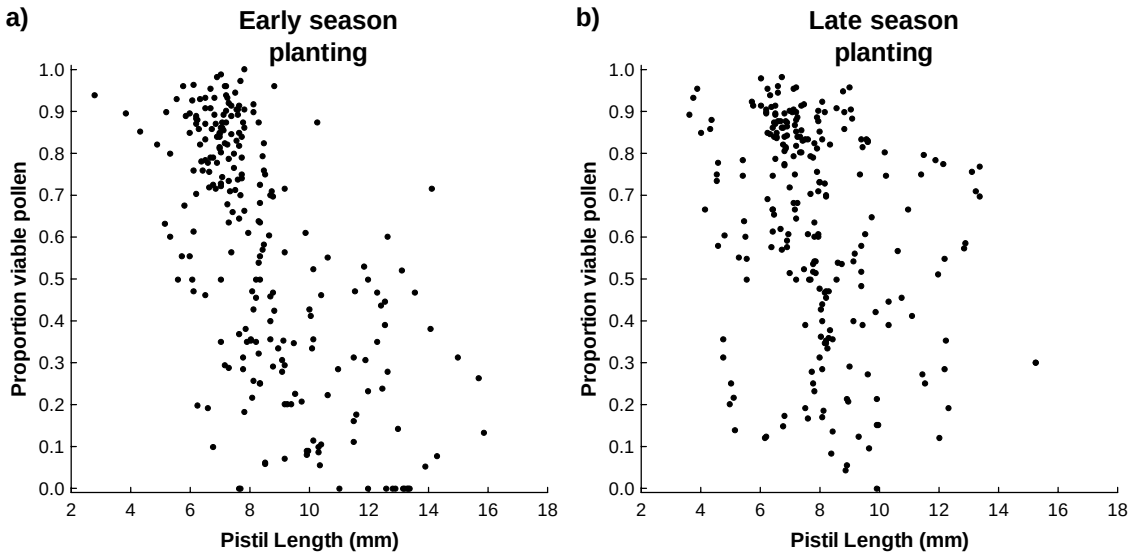


Figure 4.5 Influence of flower age, as measured by pistil length, on pollen viability of *Brassica rapa chinensis* in early season and late season crops.

Measuring the viability of pollen transported by key pollinators

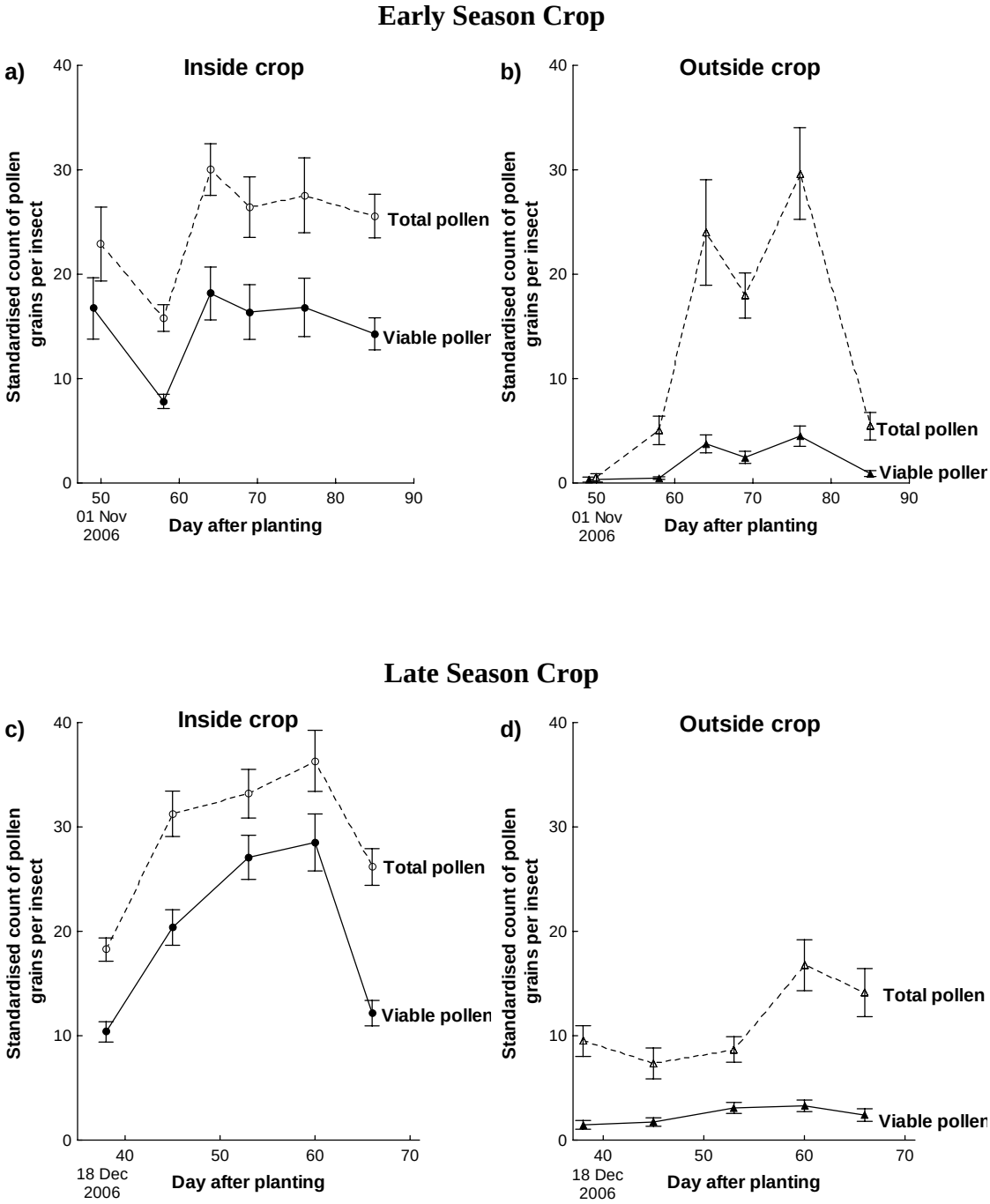


Figure 4.6 Standardised counts of total pollen and viable pollen on the bodies of five key pollinator species (combined) captured inside and outside *Brassica rapa chinensis* crops during crop development, in both early-season and late-season plantings. Values are presented as means (± 1SE) of 45 insects (nine individuals of each of five species).

Figure 4.6 shows standardised pollen counts for all five key pollinator insect species combined, in the early and the late season crop. It can be seen from the graph that in the early

season crop, total standardized counts were slightly higher inside than outside the field (Fig 4.6), but the proportion of viable pollen carried by insects was dramatically lower just 50 m outside the crop. In the late season crop, there were large difference in the total pollen load carried by insects inside and outside the crop, and pollen viability was drastically reduced outside the crop consistently with what was observed in the early season crop (Figure 4.7).

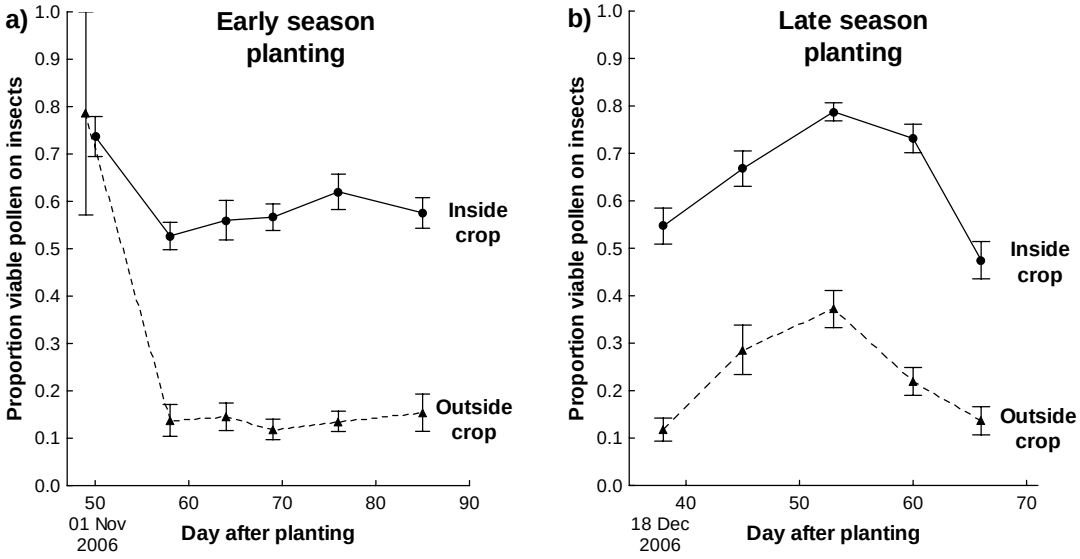
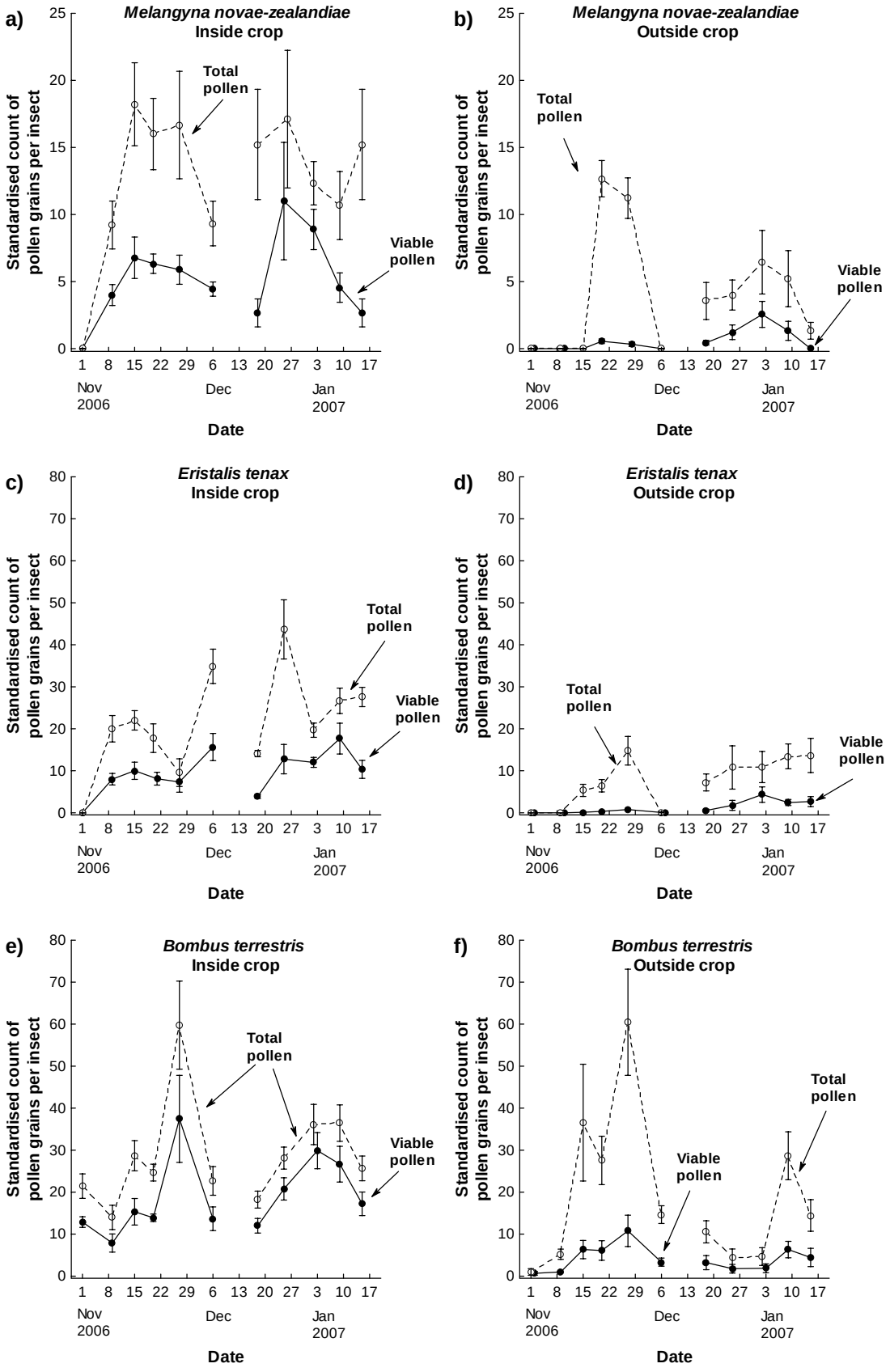


Figure 4.7 Proportion of viable pollen on key pollinators in *Brassica rapa* crops early and late in the season. Values are presented as means (± 1SE). Note that on the first two sampling dates in the early-season crop, pollen viability was assessed immediately after collection of the insects, whereas for all other sampling dates insects were stored at -80 °C for 20-40 days prior to the measurement of pollen viability.

In Figure 4.8, it can be seen that the two Diptera species carried lower amounts of pollen than the three Hymenoptera species sampled. In Diptera , *M. novae zelandiae* carried less pollen than *E. tenax*. In Hymenoptera, *L. sordidum, A. mellifera* and *B. terrestris* had similar average pollen counts. Total and viable pollen carried by insects was generally greater in the late- than in the early-season crop. There were significantly greater amounts of total pollen carried by insects inside than outside the crop for all key pollinator (P <0.007). In the case of viable pollen, all key pollinator species had significantly greater viable pollen loads inside than outside the crop ($P < 0.001$).


a)
Melangyna novae-zealandiae
Inside crop
b)
Melangyna novae-zealandiae
Outside crop
c)
Eristalis tenax
Inside crop
d)
Eristalis tenax
Outside crop
e)
Bombus terrestris
Inside crop
f)
Bombus terrestris
Outside crop
Total pollen
Viable pollen
Standardised count of pollen grains per insect
Nov 2006
Dec
Jan 2007
Date

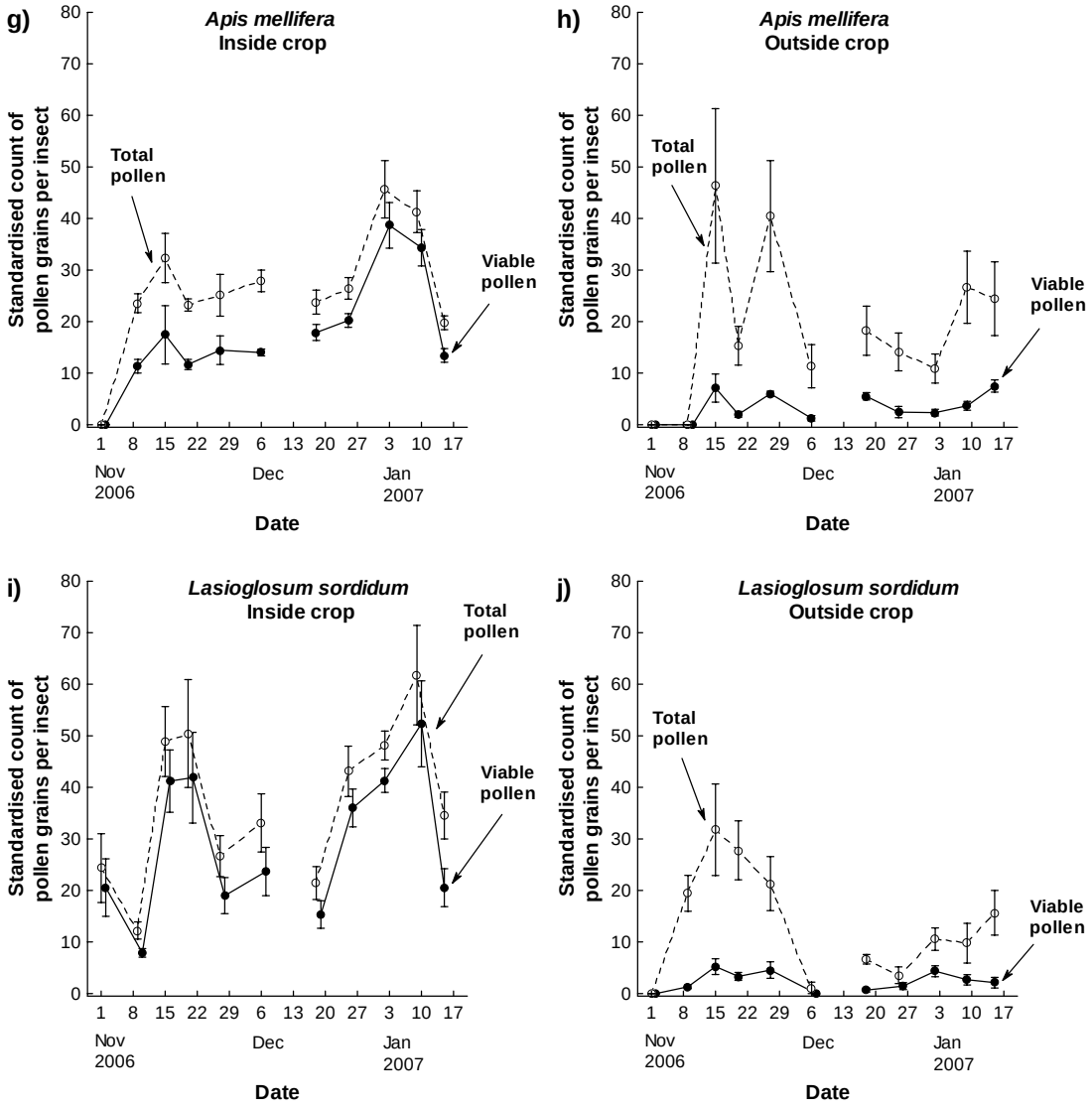


Figure 4.8. Standardised counts of total pollen and viable pollen on the body of five key pollinator species captured inside and outside *Brassica rapa chinensis* crops during crop development, in both early-season and late-season plantings. Values are presented as means (± 1SE, all $n = 9$).

Controlled experiment to test the effects of freezing preservation on pollen viability

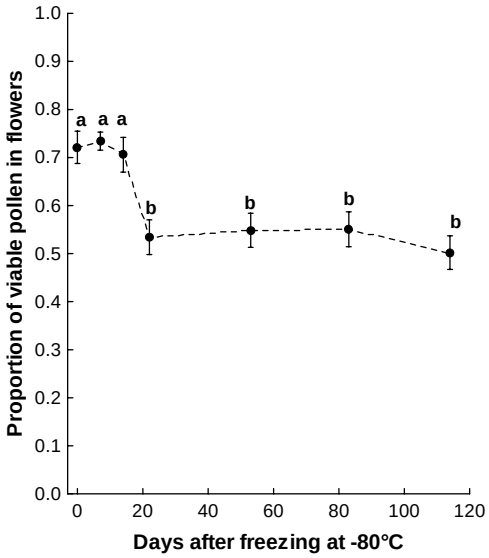


Figure 4.9 Influence of freezing time (-80 °C) on pollen viability of *Brassica rapa* in the glasshouse experiment. Values are presented as means (± 1SE) of 40 plants at each date. A Tukey test was applied to determine whether means at different dates after freezing significantly varied at $P < 0.05$.

Flowers were frozen and stored at -80 °C to test whether pollen viability decreased over time during storage. Pollen viability remained remarkably stable for the first two weeks after freezing at about 72% viable pollen, but decreased abruptly by day 20 and then remained more or less invariant at about 53% viable pollen (One-way ANOVA, $F_{6, 273} = 9.0$, $P < 0.001$, Figure 4.9).

DISCUSSION

In order to better estimate the possible role of pollen viability in gene flow, I analysed the viability of pollen in *Brassica* flowers during crop development and the viability of pollen transported by insects inside and outside one early-season crop and one late-season crop. Flower development tended to be faster and pollen viability higher in the late-season crop than in the early-season crop. Temperatures were higher in January than November at Lincoln, and therefore I speculate that increased temperature enhances flower development and pollen viability. Some authors reported that increases in temperature accelerated flower initiation and flower development (Friend 1966, 1968, Marshall & Grace 1992), and additionally that pollen transported by insects remained viable longer when temperatures

stayed between 25-30 ℃ (Bots & Mariani 2005). My results showed that flowers had total and viable pollen amounts which decreased with crop development in both the early and the late season crops. This is consistent with Pacini *et al.* (1997), who argued that pollen viability decreases as angiosperm flowers age. I also found that pollen viability correlated negatively with pistil length (as a measure of flower age). Shykoff *et al*. (1997) argue that pistil length is a good surrogate for flower aging effects on pollen viability in the anthers. Luna *et al*. (2001) reported that in maize the amount of pollen and its viability declines through the development of the crop.

Standardized pollen counts decrease slightly while pollen viability decrease sharply on insects captured 50 m from the border of the crop. Richards *et al*. (2005) argued that insects may transport pollen for up to several kilometres but that viability after such long trips is unknown. Thompson *et al*. (1999) and Morris (1995) indicated that the amount of pollen transported by insects follows a leptokurtic distribution; i.e., pollen counts show a steep decline with distance. Emberlin *et al*. (1999) found a similar relationship with insect pollen loads of maize and its viability decreasing with distance from crops, consistent with the findings of this study.

Standardized counts of pollen carried per individual were lower for the two species of Diptera than for the three species of Hymenoptera. Herrera (1987) reported greater capacity for pollen transfer of Hymenoptera than Dipera. In my study, *A. mellifera* transported more pollen than other pollinators. In contrast, Adler & Irwin (2005) reported that *Apis* carries less pollen than *Bombus* and other native pollinators of onions. I found that *L. sordidum*, *A. mellifera* and *B. terrestris* transported similar pollen loads, despite their large difference in body sizes. Similarly, Kendal & Solomon (1973) and Kendal (1973) found that solitary bees and honey bees had similar amounts of pollen with pollen viability not differing to the one found in the anther of fresh flowers of an apple trees.

There is a direct relationship between body size and pollen load carried on insect surface (Kandori 2002). Howlett (unpublished data) says that pollen loads are positively correlated with insect body size, with *A. mellifera*, *Leioproctus* sp, *Bombus* spp and *E. tenax* having the greatest amounts of pollen grains for a given body length amongst all visitors to onion crops. In contrast, Ramalho *et al* (1998) reported that in stingless bee the pollen load capacity decrease with increased body size.

Pollen grains from greenhouse *Brassica* flowers, frozen and stored at -80 °C, remained at the same level of viability as fresh pollen for up to 20 days, decreasing abruptly and significantly by approximately 20 % thereafter, after which time they remained relatively invariant in viability up to 120 days. Perveen (2007) considered that pollen is better stored at -

60°C showing 60% viability after being stored for 48 weeks. This is relevant as viability of pollen transported by insects was assessed immediately upon collection of the insects during the first sampling events (1-15 November 2006) in the early-season crop, but for the remainder of insects collected in the early- and late-season crops, pollen viability was assessed after freezing at -80 °C for 20-40 days. Therefore, the latter estimates of pollen viability may require an approximate 20% upward adjustment to account for potential preservation bias. Therefore the sudden drop in viability measured in the first two weeks in the early season crop (Figure 4.7), may be explained by preservation bias.

In conclusion, I analysed the viability of pollen in *Brassica* flowers during crop development and the viability of pollen transported by insects inside and outside one early- and one late-season crop. The study showed that pollen viability tended to decrease with crop development as measured by pistil length and that pollen viability dramatically decreased 50 m from the margin of the crop. Therefore there are greater risks of gene flow at the beginning of crop development when viability is high and insects can carry less but still viable pollen outside crops, than at the end when the majority of flowers are older sustaining low pollen viability.

CHAPTER FIVE

SYNTHESIS

The broad aim of this thesis was to examine how spatial and temporal variation in the abundance and behavioural activity of key insect pollinator species contributes to gene flow into and out of *Brassica rapa* crops. I looked at variation in the abundance of flower visitors and the foraging behaviour of key pollinators during crop development, and also the viability of pollen in *Brassica* flowers and insects inside and outside the crops. The methods involved periodically measuring crop development, measuring insect abundance using window traps, following individual insects for three minutes to assess pollinator behaviour and assessing pollen viability using a fluorescence technique. The most common visitors were from Diptera and Hymenoptera and followed crop development with greatest insect abundance at the peak of flowering. Not uncommonly more insects were attracted into the crop early in the season, staying there rather than leaving, and then when flowers started to disappear there was massive escape of insects from the crop. Insect abundance and pollen viability sharply decreased 50 m from the margin of the crop but still to levels that represent a threat to gene flow. Also pollen viability decreased with crop development and pistil length. I found species specific differences in the behaviour of key pollinators with crop development, with *B. terrestris* showing the greatest rate and variability of movement. Therefore greater risks for gene flow are posed early on crop development when pollen viability is greatest, and such risks are differentially affected by distinct pollinator species.

The first objective for this thesis was to assess variation in pollinator diversity and relative abundance during crop development, contrasting capture rates and directional movement inside and 50 m outside the crop border. I found that capture rates were greater in the early than in the late season crop, and the abundance at the family level for both crops studied was similar. Across flowering development, I observed that the largest numbers of insects were captured at the peak of flowering for both crops. During the flowering period, Diptera was the most abundant order collected, followed by Hymenoptera. The numbers of insects captured 50 m from the edge of the crop were 10% and 33% of the total captured inside the crop, for the early- and late-season crops, respectively. This is relevant as the risk of gene flow depends on insects carrying pollen outside the crop, and these proportions show that cross-pollination is highly feasible. The proportion of insects entering versus leaving the

crop varied considerably across species, crops and spatial location of traps inside or 50 m outside the border of the crop, but not uncommonly more insects entered early on crop development switching to more insects leaving late in the season.

The second objective for this thesis was to evaluate species-specific variation in the behavioural activities of the five most important flower visitors to *Brassica rapa chinensis* throughout the crop flowering cycle. This research highlights the influence of crop age on the foraging behaviour of key pollinators and the species-specific dissimilarity in the foraging behaviours of *Brassica* visitors with crop development. Temporal variation in the rate and variability of movement between flowers, and the duration and variability in time spent on each flower, throughout the growing season, differed markedly between pollinator species. Flower density, plant density, and the abundance of other insect pollinators contributed in explaining pollinator behaviour for *A. mellifera*, *E. tenax*, *M. novae-zelandiae* and *L. sordidum*. This study showed that as plant density and open flower density increased, the average flower visitation rate increased for most species, while the average distance flown between flowers decreased significantly. One key finding of this study was that *Bombus terrestris* had the greatest rates and variability of movement, and the greatest rates of flower visitation among all key pollinators studied. Therefore *B. terrestris* might contribute to gene flow to a greater extent than other key pollinators as shown by previous research (Waddington 1981, Rasmussen & Brodsgaard 1992, Walklate *et al*. 2004).

The third objective for this thesis was to examine how total pollen loads and the viability of pollen carried by five key pollinator species inside and outside the crop varied with flower phenology throughout the growing season. I observed that total and viable pollen in flowers tended to decrease with crop development in both the early and the late season crops. Standardized total pollen counts on insects captured 50 m from the border of the crop were slightly lower than on insects captured inside the crop, but pollen viability decreased sharply but still to levels that may represent risks for gene flow. I found that *L. sordidum*, *A. mellifera* and *B. terrestris* transported similar pollen loads, which were much greater than those carried by *E. tenax* and *M. novae-zelandiae*. This might be explained because the former species have specific adaptations to concentrate pollen into their “leg scopae” and also they possess a body and a head covered with hair that increases their capacity for pollen transport.

In summary, I examined how spatial and temporal variation in the abundance and behavioural activity of key insect pollinator species contribute to gene flow into and out of *Brassica rapa* crops. I found that risks of gene flow depend on crop development and pollinator species. Greater risks of gene flow were associated to early on crop development because is at this stage that pollen viability is greatest decreasing progressively towards the end of the season. Pollen viability decreased sharply in insects captured 50 m from the border of the crop but still to levels that may represent a significant threat for gene escape from crops. I found dissimilar behaviour among the key pollinators studied both in scale and in the response of behavioural metrics to crop development. Flower density, plant density, and pollinator abundance successfully contributed in explaining pollinator behaviour in all but *B. terrestris*. *B. terrestris* was atypical in that did not respond to crop development and showed far greater rates and variability of movement and flower visitation than other pollinators, and may therefore significantly contribute to gene escape from crops.

In order to better understand the influence of pollinator diversity and behaviour on pollen movement in *Brassica rapa chinensis* (Pak-Choi) crops, and its significance for gene escape further research is needed. Further research would require to better know the distribution of total and viable pollen within the insect body, to assess pollen deposition on virgin stigmas after single visits, to understand the relationship between body size and pollen viability, to assess the sustainability of pollination services by native bees excluding exotic pollinators, to extend pollen transport and viability assessment over a gradient of several kilometres, to find the relationship between nectar production and pollinator activity and to better understand the role of plant density, flower density and pollinator abundance on pollinator behaviour, among others.

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Appendix 2.1. A comparison of the relative abundances of insects captured in directional window traps placed both inside and outside of an early-season planting and a late-season planting of *Brassica rapa chinensis* in Lincoln, Canterbury, New Zealand. 'Entering' and 'Leaving' indicate the direction of movement of the insects when they were captured in the window trap. For each species, in each of the two crops, a χ^2 goodness of fit test was used to determine whether the frequency of individuals entering versus leaving the crop differed significantly between window traps placed inside and outside the crop. Bolded *P*-values are significant at $P < 0.05$.

	Early-season planting						Late-season planting					
	Inside		**Outside**				**Inside**		**Outside**			
	Entering	**Leaving**	**Entering**	**Leaving**	***χ2***	***p***	**Entering**	**Leaving**	**Entering**	**Leaving**	***χ2***	***p***
Coleoptera												
Costelytra zealandica	148	143	108	85	1.210	0.271	1	1	2		1.330	0.249
Coccinella undecimpunctata	1			2	3.000	0.083	37	46	31	31	0.420	0.517
Diptera												
Tachinidae sp 1	29	49	10	8	2.050	0.152	47	26	8	20	10.470	**<0.001**
Anthomyia punctipennis								1	3	2	1.200	0.273
Beris spp.	3547	3578	52	64	1.120	0.290	1663	1786	147	131	2.240	0.134
Calliphora stygia							2	1	1		0.440	0.507
Calliphora vicinia	13	20	9	14	0.000	1.000	5	3		1	1.410	0.235
Delia platura		3					101	172	50	31	15.620	**<0.001**
Dilophus nigrostigma	17	15	3	1	0.690	0.435	73	100	1		1.360	0.244
Ephydridae	39	32	30	34	0.870	0.351						
Eristalis tenax	1						4	4		14	8.560	**<0.003**
Helophilus hochstetteri							14	6	1	12	12.340	**<0.001**
Lucilia sericata	2	4					2	1		1	1.330	0.249
Megathereva bilineata		4	11	7	4.890	**0.027**	2		1			
Melangyna novae-zelandiae	4	4					4	4	9	18	0.730	0.393
Melanostoma fasciatum	8	5		3	3.690	0.055	4	3	3	10	2.320	0.128
Oxysarcodexia varia							31	26	24	35	2.180	0.140
Scaptia adrel	230	257	17	6	6.260	**0.012**	1	1				
Hemiptera												
Arocatus rusticus		1	2	2	0.830	0.362						
Hymenoptera												

Apis mellifera	179	196	39	29	2.130	0.194	228	237	57	51	0.490	0.484
Bombus terrestris	136	121	76	80	0.690	0.406	48	61	11	19	0.520	0.471
Ichneumonidae	4	2	4		1.670	0.413	5	2	4		1.400	0.237
Lasioglossum ssp							67	72	350	480	1.770	0.183
Leioproctus spp	66	71	70	62	0.630	0.427	4	4	3	24	5.830	**<0.016**
Vespula germanica		1					5	1	2		0.380	0.538
Lepidoptera												
Moths	46	49	48	47	0.080	0.777	48	28	15	26	7.570	**<0.006**
Pieris rapae	1						1		2	12	4.290	**<0.038**
Neuroptera												
Kempynus incisus	9	2	6	3	0.610	0.435	5	7	10	5	1.690	0.194
Grand Total	4480	4557	485	447	2.050	0.152	2402	2593	735	923	7.050	**<0.008**

Appendix 2.2. A comparison of the ratios of key insect pollinator species captured entering versus leaving directional window traps placed both inside and outside fields of early and late season plantings of *Brassica rapa chinensis* in Lincoln, Canterbury, New Zealand.

	Early-season planting		**Late-season planting**	
	Inside	**Outside**	**Inside**	**Outside**
Apis mellifera	1.28 (0.53 - 3.20)	1.61 (0.58 - 2.50)	0.95 (0.55 - 1.30)	1.30 (0.46 - 2.71)
Bombus terrestris	1.12 (0.83 - 1.86)	1.29 (0.36 - 3.50)	0.80 (0.60 - 1.23)	0.80 (0.45 - 1.00)
Lasioglossum sordidum	1.00 (1.00 - 1.00)	1.00 (1.00 - 1.00)	0.92 (0.42 - 1.93)	0.81 (0.48 - 1.12)
Eristalis tenax	1.14 (1.00 - 2.00)	1.00 (1.00 - 1.00)	1.00 (0.67 - 1.33)	0.68 (0.08 - 1.00)
Melangyna novae-zealandiae	1.00 (0.50 - 1.50)	1.00 (1.00 - 1.00)	1.20 (0.50 - 3.00)	0.81 (0.50 - 1.00)

Appendix 3.1. Comparison of main behavioural variables of five key pollinators in a *Brassica rapa* crop in Canterbury, New Zealand. Values are presented as means (± 1 SE).

Days after planting	Flower density (Open flowers m^{-2})	Rate of Movement (cm min^{-1})	Variability of movement (%)	Rate of flower visitation (flow. min^{-1})	Variability of flower visitation (%)
M. novae-zelandiae					
52	13	634 ± 51	0.58 ± 0.03	17.9 ± 0.7	0.49 ± 0.01
55	158	239 ± 29	0.76 ± 0.04	7.6 ± 0.4	0.36 ± 0.02
63	2683	232 ± 32	1.19 ± 0.05	8.2 ± 0.9	0.47 ± 0.05
69	4508	137 ± 25	1.09 ± 0.10	8.0 ± 1.0	0.77 ± 0.09
76	7118	129 ± 21	0.93 ± 0.09	7.5 ± 0.5	0.86 ± 0.05
81	656	69 ± 7	1.06 ± 0.09	4.5 ± 0.3	0.86 ± 0.06
Average		**240 ± 28**	**0.93 ± 0.04**	**8.9 ± 0.6**	**0.64 ± 0.04**
E. tenax					
52	13	399 ± 50	1.24 ± 0.11	12.0 ± 0.6	0.43 ± 0.04
55	158	284 ± 32	1.29 ± 0.11	13.8 ± 0.9	0.40 ± 0.04
63	2683	203 ± 20	1.42 ± 0.08	11.9 ± 0.6	0.56 ± 0.06
69	4508	142 ± 14	1.27 ± 0.11	16.5 ± 0.7	0.31 ± 0.01
76	7118	201 ± 25	1.38 ± 0.11	13.5 ± 0.9	0.44 ± 0.04
81	656	156 ± 21	1.16 ± 0.12	11.7 ± 0.8	0.47 ± 0.03
Average		**240 ± 16**	**1.29 ± 0.04**	**13 ± 0.4**	**0.44 ± 0.02**
B. terrestris					
52	13	396 ± 35	1.20 ± 0.23	16.2± 0.8	0.39 ± 0.03
55	158	706 ± 110	1.22 ± 0.13	14.0 ± 0.6	0.40 ± 0.01
63	2683	525 ± 75	1.63 ± 0.18	31.1 ± 1.8	0.50 ± 0.04
69	4508	373 ± 30	1.62 ± 0.31	35.6 ± 0.9	0.48 ± 0.02
76	7118	454 ± 38	0.90 ± 0.07	21.1 ± 0.9	0.41 ± 0.03
81	656	385 ± 41	1.81 ± 0.38	30.8± 1.1	0.43 ± 0.03
Average		**473 ± 29**	**1.40 ± 0.10**	**25.0 ± 1.2**	**0.43 ± 0.01**
A. mellifera					
52	13	585 ± 75	1.31 ± 0.10	12.0 ± 0.9	0.69 ± 0.14
55	158	420 ± 55	1.07 ± 0.06	12.7 ± 0.5	0.40 ± 0.02
63	2683	359 ± 32	1.37 ± 0.09	12.4 ± 0.3	0.44 ± 0.04
69	4508	184 ± 15	0.95 ± 0.11	18.6 ± 0.4	0.32 ± 0.04
76	7118	229 ± 15	1.21 ± 0.11	16.0 ± 0.8	0.36 ± 0.03
81	656	243 ± 16	1.26 ± 0.17	17.2 ± 0.4	0.32 ± 0.02
Average		**336 ± 25**	**1.19 ± 0.05**	**14.8 ± 0.4**	**0.42 ± 0.03**
L. sordidum					
52	13	111 ± 17	0.94 ± 0.05	2.9 ± 0.4	0.88 ± 0.08
55	158	121 ± 12	0.94 ± 0.06	5.5 ± 0.5	0.72 ± 0.08
63	2683	108 ± 15	1.01 ± 0.06	7.4 ± 0.4	0.61 ± 0.07
69	4508	67 ± 15	1.16 ± 0.22	5.1 ± 0.1	0.91 ± 0.09
76	7118	129 ± 16	1.15 ± 0.07	6.1 ± 0.5	0.64 ± 0.03
81	656	45 ± 4	0.99 ± 0.05	3.1 ± 0.2	0.75 ± 0.03
Average		**97 ± 7**	**1.03 ± 0.04**	**5.1 ± 0.3**	**0.75 ± 0.03**

Appendix 3.2. General Linear Models testing whether the five key pollinator species (*M. novae-zelandiae*, *E. tenax*, *B. terrestris*, *A. mellifera* and *L. sordidum*) exhibited different behavioural responses with crop development.

a) Logarithm of rate of movement

	d.f.	SS	MS	F	P
Days after planting	5	30.713	6.143	47.73	<0.001
Species	4	83.393	20.848	162.01	<0.001
Species * Days after planting	20	18.801	0.940	7.31	<0.001
Error	240	30.884	0.129		
Total	269	163.791			

b) Logarithm of rate of flower visitation

	d.f.	SS	MS	F	P
Days after planting	5	3.407	0.681	15.86	<0.001
Species	4	81.420	20.355	473.95	<0.001
Species * Days after planting	20	21.311	1.066	24.81	<0.001
Error	240	10.307	0.043		
Total	269	116.444			

c) Logarithm of variability of movement

	d.f.	SS	MS	F	P
Days after planting	5	2.183	0.437	5.319	<0.001
Species	4	4.935	1.234	15.027	<0.001
Species * Days after planting	20	4.862	0.243	2.961	<0.001
Error	240	19.703	0.082		
Total	269	31.683			

d) Logarithm of variability of flower visitation

	d.f.	SS	MS	F	P
Days after planting	5	1.143	0.229	3.737	<0.003
Species	4	15.068	3.767	61.572	<0.001
Species * Days after planting	20	10.665	0.533	8.716	<0.001
Error	240	14.683	0.061		
Total	269	41.559			

Appendix 3.3. General Linear Models testing the variation in four selected behavioural response parameters of five key pollinator species (*M. novae-zelandiae, E. tenax, B. terrestris, A. mellifera* and *L. sordidum*) in relation to varying crop phenology (Days after planting), after first taking into account significant microclimatic variation at the time of day that individual insects were sampled. Variance decomposition analysis was used to determine the proportion of variation in the Day after planting effect that was due to the co-varying effects of plant density, flower density and pollinator abundance.

a) Logarithm of rate of movement

Melangyna novae-zelandiae

Source	d.f.	S.S.	M.S.	*F*	*P*
Wind speed	1	2.750	2.750	14.829	<0.001
Temperature	1	1.130	1.130	6.092	<0.017
Light	1	10.289	10.289	55.484	<0.001
Days after planting	5	12.870	2.574	13.881	<0.001
Plant density	1	7.038	7.038	8.886	
Flower density	1	4.199	4.199	5.302	
Pollinator abundance	1	0.049	0.049	0.062	
Error within Days after planting	2	1.584	0.792		
Error	45	8.345	0.185		
Total	53	35.384			

Eristalis tenax

Source	d.f.	S.S.	M.S.	*F*	*P*
Wind speed	1	1.437	1.437	12.600	<0.001
Light	1	0.553	0.553	4.850	0.033
Temperature	1	1.210	1.210	10.620	<0.001
Days after planting	5	2.452	0.490	4.301	0.003
Plant density	1	1.197	1.197	2.071	
Flower density	1	0.060	0.060	0.104	
Pollinator abundance	1	0.039	0.039	0.067	
Error within Days after planting	2	1.156	0.578		
Error	45	5.131	0.114		
Total	53	10.784			

Bombus terrestris

Source	d.f.	S.S.	M.S.	*F*	*P*
Relative humidity	1	0.791	0.791	8.35	0.006
Wind speed	1	0.515	0.515	5.44	0.024
Days after planting	5	1.036	0.207	2.19	0.072
Error	46	4.356	0.095		
Total	53	6.697			

Apis mellifera

Source	d.f.	S.S.	M.S.	*F*	*P*
Wind speed	1	1.146	1.146	12.160	<0.001
Temperature	1	1.477	1.477	15.680	<0.001
Light	1	0.642	0.642	6.810	0.012
Days after planting	5	4.368	0.874	9.270	<0.001
Plant density	1	0.816	0.816	45.333	
Flower density	1	3.379	3.379	187.722	
Pollinator abundance	1	0.138	0.138	7.667	
Error within Days after planting	2	0.035	0.018		
Error	45	4.239	0.094		
Total	53	11.872			

Lasioglossum sordidum

Source	d.f.	S.S.	M.S.	*F*	*P*
Relative humidity	1	2.095	2.095	14.674	<0.001
Light	1	3.418	3.418	23.939	<0.001
Days after planting	5	3.581	0.716	5.015	<0.001
Plant density	1	0.629	0.629	0.718	
Flower density	1	0.180	0.180	0.205	
Pollinator abundance	1	1.021	1.021	1.166	
Error within Days after planting	2	1.751	0.876		
Error	46	6.568	0.143		
Total	53	15.662			

b) Logarithm of rate of flower visitation

Melangyna novae-zelandiae

Source	d.f.	S.S.	M.S.	*F*	*P*
Light	1	5.939	5.939	86.318	<0.001
Temperature	1	0.630	0.630	9.157	0.004
Days after planting	5	2.667	0.533	7.752	<0.001
Plant density	1	2.033	2.033	7.286	
Flower density	1	0.008	0.008	0.029	
Pollinator abundance	1	0.068	0.068	0.244	
Error within Days after planting	2	0.558	0.279		
Error	46	3.165	0.069		
Total	53	12.399			

Eristalis tenax

Source	d.f.	S.S.	M.S.	*F*	*P*
Temperature	1	0.149	0.149	4.76	0.034
Light	1	0.197	0.197	6.27	0.016
Days after planting	5	0.569	0.114	3.63	0.008
Plant density	1	0.155	0.155	1.383	
Flower density	1	0.139	0.139	1.241	
Pollinator abundance	1	0.050	0.051	0.455	
Error within Days after planting	2	0.223	0.112		
Error	46	1.442	0.031		
Total	53	2.358			

Bombus terrestris

Source	d.f.	S.S.	M.S.	*F*	*P*
Wind speed	1	2.877	2.877	182.57	<0.001
Light	1	1.464	1.464	92.87	<0.001
Temperature	1	2.183	2.183	138.49	<0.001
Relative humidity	1	0.228	0.228	14.48	<0.001
Days after planting	5	0.183	0.037	2.32	0.059
Error	44	0.694	0.016		
Total	53	7.628			

Apis mellifera

Source	d.f.	S.S.	M.S.	*F*	*P*
Wind speed	1	0.423	0.423	20.880	<0.001
Relative humidity	1	0.221	0.221	10.890	<0.001
Light	1	0.278	0.278	13.700	<0.001
Temperature	1	0.245	0.245	12.100	<0.001
Days after planting	5	0.574	0.115	5.66	<0.001
Plant density	1	0.151	0.151	25.166	
Flower density	1	0.397	0.397	66.166	
Pollinator abundance	1	0.013	0.013	2.166	
Error within Days after planting	2	0.013	0.006		
Error	44	0.891	0.020		
Total	53	2.631			

Lasioglossum sordidum

Source	d.f.	S.S.	M.S.	*F*	*P*
Temperature	1	0.695	0.695	10.336	<0.001
Relative humidity	1	1.977	1.977	29.415	<0.001
Light	1	0.962	0.962	14.313	<0.001
Wind speed	1	0.726	0.726	10.802	<0.001
Days after planting	5	2.693	0.539	8.016	<0.001
Plant density	1	0.492	0.492	1.329	
Flower density	1	0.003	0.003	0.008	
Pollinator abundance	1	0.458	0.458	1.238	
Error within Days after planting	2	1.740	0.370		
Error	44	2.957	0.067		
Total	53	10.008			

c) Logarithm of variability of movement

Melangyna novae-zelandiae

Source	d.f.	S.S.	M.S.	*F*	*P*
Light	1	1.427	1.427	32.607	<0.001
Relative humidity	1	0.842	0.842	19.225	<0.001
Days after planting	5	1.048	0.209	4.789	<0.001
Plant density	1	0.420	0.420	1.714	
Flower density	1	0.100	0.100	0.408	
Pollinator abundance	1	0.036	0.036	0.147	
Error within Days after planting	2	0.491	0.245		
Error	46	2.013	0.043		
Total	53	5.330			

Eristalis tenax

Source	d.f.	S.S.	M.S.	*F*	*P*
Relative humidity	1	0.348	0.348	5.612	0.022
Error	52	3.082	0.059		
Total	53	3.430			

Bombus terrestris

Source	d.f.	S.S.	M.S.	*F*	*P*
Light	1	0.956	0.956	5.131	0.028
Error	52	9.687	0.186		
Total	53	10.642			

Apis mellifera

Source	d.f.	S.S.	M.S.	*F*	*P*
Light	1	0.425	0.425	6.216	0.016
Error	52	3.559	0.068		
Total	53	3.985			

Lasioglossum sordidum

No variable explained significant variability of movement in *L. sordidum*.

d) Logarithm of variability of flower visitation

Melangyna novae-zelandiae

Source	d.f.	S.S.	M.S.	*F*	*P*
Wind speed	1	0.461	0.461	6.513	0.014
Days after planting	5	5.518	1.103	15.578	<0.001
Plant density	1	1.064	1.064	1.009	
Flower density	1	2.301	2.302	2.182	
Pollinator abundance	1	0.041	0.042	0.040	
Error within Days after planting	2	2.110	1.055		
Error	47	3.329	0.071		
Total	53	9.308			

Eristalis tenax

Source	d.f.	S.S.	M.S.	*F*	*P*
Days after planting	5	1.429	0.286	5.031	<0.001
Plant density	1	0.291	0.291	0.536	
Flower density	1	0.045	0.045	0.008	
Pollinator abundance	1	0.008	0.008	0.015	
Error within Days after planting	2	1.084	0.543		
Error	48	2.726	0.056		
Total	53	4.155			

Bombus terrestris

Source	d.f.	S.S.	M.S.	*F*	*P*
Light	1	0.287	0.286	9.835	0.003
Days after planting	5	0.234	0.046	1.599	0.178
Error	47	1.371	0.029		
Total	53	1.891			

Apis mellifera

Source	d.f.	S.S.	M.S.	*F*	*P*
Wind speed	1	0.502	0.502	5.876	0.019
Days after planting	5	2.391	0.478	5.601	<0.001
Plant density	1	1.357	1.357	6.652	
Flower density	1	0.589	0.589	2.887	
Pollinator abundance	1	0.036	0.036	0.176	
Error within Days after planting	2	0.408	0.204		
Error	47	4.012	0.085		
Total	53	6.905			

Lasioglossum sordidum

Source	d.f.	S.S.	M.S.	*F*	*P*
Wind speed	1	0.309	0.309	4.786	0.034
Days after planting	5	0.885	0.177	2.742	0.029
Plant density	1	0.396	0.396	1.792	
Flower density	1	0.046	0.045	0.204	
Pollinator abundance	1	0.002	0.002	0.009	
Error within Days after planting	2	0.441	0.221		
Error	47	3.036	0.064		
Total	53	4.230			